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Aerobic Sporeforming Bacteria

U. S. DEPARTMENT OF AGRICULTURE

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Aerobic Sporeforming Bacteria

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[3] The writers wish to express their appreciation to the many individuals and organizations that generously supplied cultures used in this study. This monograph supersedes Miscellaneous Publication 559, Aerobic Mesophilic Sporeforming Bacteria.

SUMMARY

AEROBIC SPOREFORMING BACTERIA are usually considered as inhabitants of the soil. Because of the formation of heat-resisting spores, they may be found practically anywhere. They may cause spoilage of food and other materials, and, because of their strong enzymatic activity, they are used in certain industrial fermentations. Various infections in the honey bee and other insects and anthrax in warm-blooded animals can be attributed to them. Since 1940 much interest in this genus has arisen because of the discovery of antibiotic substances produced by certain species.

Ford and his coworkers attempted to classify this genus in 1916, but lack of knowledge of the different stages of growth and the variation in bacterial cultures, as well as lack of adequate differential tests, left their work incomplete. The result was that bacteriologists—medical and agricultural—often attached a new name to a culture they had isolated, believing it to be a new species. This, combined with the distribution of mislabeled cultures, led to such confusion that no one could identify with certainty more than a very few of the species.

A total of 1,134 cultures of aerobic sporeforming bacteria were studied by the writers and 1,114 were placed in 3 groups on the basis of morphology and physiology. Of the total, 491 bore specific names; the remaining 643 either were received unnamed or were isolated by the writers.

Of the 3 groups, the first is the largest, comprising about 65 percent of the cultures studied. It includes *Bacillus megaterium*, *B. cereus* and varieties, *B. licheniformis*, *B. subtilis* and varieties, *B. pumilus* (formerly called *B. mesentericus* in America), and the lesser known species *B. coagulans*, *B. firmus*, and *B. lentus*. Studies on dissociation were made with *B. mycoides*, which resulted in its being listed as a variety of *B. cereus*. Comparative studies indicated that *B. anthracis* differed from *B. cereus* in being pathogenic and nonmotile. As both are mutable characters, *B. anthracis* has been placed as a variety of *B. cereus*. Dissociation of the black-pigment-producing species *B. niger* and *B. aterrimus* to colorless forms indistinguishable from *B. subtilis* proved that they were only varieties of the latter.

Group 2 contains about 30 percent of the cultures. It includes *B. polymyxa*, *B. macerans*, *B. stearothermophilus*, *B. circulans*, *B. alvei*, *B. laterosporus*, *B. pulvifaciens*, and *B. brevis*. Of these, *B. circulans* was the most difficult to characterize, because of great variations among the cultures. It seems to represent a complex that may eventually be divided into varieties, or even into species if additional stable characters are found. Its close relationship with *B. macerans*, on the one hand, and with *B. stearothermophilus*, on the other, was especially noteworthy. *Bacillus stearothermophilus* was the only species capable of growth at 65° C. or higher.

Group 3, the round-spored group, with only about 5 percent of the cultures, contains *B. pantothenticus*, *B. sphaericus*, and *B. pasteurii*. Of the 3 varieties formerly assigned to *B. sphaericus*, one (var. *fusiformis*) is now considered as a urease-positive variant, whereas the other two (var. *rotans* and var. *loehnisii*) have been placed in the list of unclassified cultures.

The Voges-Proskauer reaction proved an important and a reliable test for separating some species if certain modifications of the usual method were followed. The storage of fat and the fermentation of carbon compounds were studied extensively and were found valuable, provided liberal interpretations were made of the observations. Of special interest was the ability of certain strains to produce adaptive enzymes, so that carbohydrates not regularly attacked could be utilized. In many cases these cultures lost this acquired ability after a few months' storage on nutrient agar in the absence of the carbohydrate.

Agglutination by antiserums produced with whole cells as antigens was too specific for taxonomic use. Bacteriophage, although too specific in some instances, was very useful in others, especially in the study of *B. megaterium*, *B. cereus* and varieties, *B. pumilus*, and *B. brevis*.

It was found that many species previously described and separated from others because of a rough, folded, mucoid, rhizoid, or pigmented growth were merely growth phases or varieties of established species. It was also found that still other species characterized by some special physiological trait were only biotypes of a parent species. Consequently, a large number of named cultures have been placed in the synonymy of the various species. If the original description was not adequate to characterize the organism, it was assigned to a species on the basis of the culture as received.

A dichotomous key for use in identifying cultures of the aerobic sporeforming bacteria has been prepared in which two characters, so far as possible, are used at each separation instead of one.

NEED FOR CLASSIFICATION

After Ford and his associates (*93, 94*)[4] published their work on the sporeforming bacteria very little progress was made for 30 years in the classification of this group. During that time new species were described briefly without comparing them with those already mentioned in the literature. Often there was only a single isolation to which was ascribed some special character, such as the ability to grow upon a certain medium or to produce a certain byproduct, and no attempt was made to observe closely related strains for the same property. For a number of years it was obvious that this genus was poorly arranged and needed thorough study. The stimulus to attempt this came when a culture of

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[4] Italic numbers in parentheses refer to Literature Cited, p. 134.

Bacillus siamensis Siribaed (*144*) was received and identified as a strain of *B. cereus* (*28*).

As the best method of approach seemed to be by an investigation of authentic cultures, requests for transplants of various members of the genus were sent to individuals, laboratories, and institutions, resulting in a collection of 363 named strains and 258 unnamed cultures and isolations. These were studied and the results published in 1946 (*147*). Since the completion of that work 128 additional named strains and 385 unidentified cultures, including 167 capable of growing at high temperatures, have been obtained. Examination of these newly acquired strains and continued study of the original collection necessitated the characterization of 2 more species (*B. licheniformis* and *B. stearothermophilus*) and certain changes in some of the descriptions published in 1946. In addition, 2 new species (*B. pulvifaciens* and *B. pantothenticus*) presented by other workers are included.

As the number of strains in the collection increased and information about the cultures accumulated it became evident that certain characters were comparatively stable, whereas others were so variable as to be taxonomically useless. The limits of variability in certain characters have been established for some species. This has resulted in the discard of some old specific names of the genus *Bacillus* and in the demotion of other species to the rank of varieties or biotypes.

PROBLEMS IN CLASSIFYING BACTERIA

It is common knowledge that cultures may pass through various growth stages (rough, smooth, mucoid, dwarf, and other stages) and may vary in pigment formation, carbohydrate fermentation, and, to a lesser extent, even as to pathogenicity. In fact, it is believed by an increasing number of bacteriologists that any character may vary to some extent. It becomes necessary, therefore, to rely on a group of properties for specific differentiation, or what has been called a species pattern. The pattern of one species may overlap that of a closely related species, giving rise to intermediate cultures. One should not, however, be able to change a culture from one pattern to another; in other words, cultures of one species should not change into those of another, either spontaneously or by changes induced in the laboratory. The classification proposed in this monograph is based on such a stable species concept. In some cases justification for the arrangement has been obtained by studies on dissociation and variation in the cultures; in others, by the presence of cultures in the collection that represented certain stages of growth or variations in physiology of stable parent species.

The choice of a name presents a problem when a species pattern covers several named species that are now reduced to varieties or dissociants. One solution would be to select the name that appeared first in the literature (priority) without regard to systematic relations or the stability of the characters.

Another solution, presented here, puts the natural relations of the dissociants and variants ahead of priority when it seems necessary in the development of the classification. It is based on the concept of a stable basic or parent species that in turn is supported by observations on the morphological and physiological variations of these organisms. Some examples will illustrate what is meant, the first one, by chance, not being at variance with priority.

The species pattern of *Bacillus niger* was found in a previous publication (*31*) to be identical with that of *B. subtilis* except for the ability of *B. niger* to blacken mediums containing tyrosine. Cultures of *B. niger* were dissociated to the nonpigmented stage that could not be distinguished from *B. subtilis*. (See Studies on Dissociation.) Another species, *B. aterrimus*, was also observed to have the same species pattern as *B. subtilis* except for its formation of a black pigment from carbohydrates (*31*). Nonpigment-producing dissociants of strains of *B. aterrimus* were also obtained and could not be separated from *B. subtilis*. These induced variants had their counterparts in the writers' collection in two strains named *B. niger* and four labeled *B. aterrimus* that have not blackened the proper mediums during this investigation. These six strains, named because of their pigments, apparently reverted later to their stable parent species, *B. subtilis*. It is assumed, therefore, that *B. subtilis* has given rise, sometime in the course of evolution, to two different pigment-forming varieties that will by spontaneous or induced dissociation lose their differentiating character of pigmentation.

It is believed that the classification, or naming, of these three species should express their natural relationship and that this can best be done by retaining the name of the parent, or more stable, species—in this case *B. subtilis*—and by listing the other two as varieties. A strain of *B. subtilis* var. *aterrimus*, for example, that had lost its pigment would still have the species pattern of *B. subtilis* and would keep that species name. Only its varietal name would be dropped. If, under certain conditions as yet unknown, a strain of *B. subtilis* could be induced to blacken tyrosine mediums, its species' name would remain unchanged and merely the designation, var. *niger*, need be added.

According to the International Bacteriological Code of Nomenclature (*16, p. 294*) a "variant is an organism showing some variation in some character from the parent culture" and "if sufficiently distinct and stable" the organism should be "regarded and named as a subspecies or variety." If these definitions were applied in the above-mentioned dissociation studies and if *B. niger* were the parent culture, the non-pigment-forming dissociant, *B. subtilis,* would be the variety. The untenable designation of *B. subtilis* as a variety of two different species would be further complicated by the strains of *B. subtilis* that have no known connection with the two pigment-producing species.

Bacillus cereus and its closely allied forms also provide excellent support for the concept of a stable parent species and its varieties. This relationship is illustrated in figure 1.

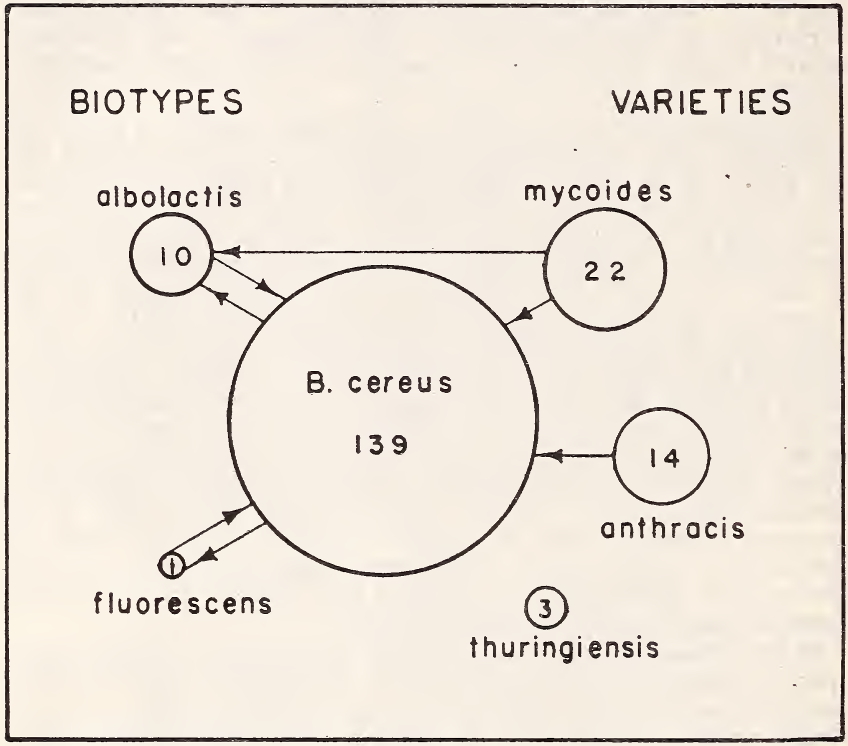

Figure 1.—*Bacillus cereus*, with its varieties and biotypes, and the changes (indicated by arrows) observed to take place. Most of the changes were induced in the laboratory, but some were spontaneous. The area of each circle is equal to the number of cultures studied multiplied by 10 mm.²

Each of the surrounding so-called species, *B. albolactis*, *B. fluorescens*, *B. mycoides*, *B. anthracis*, and *B. thuringiensis*, proved to have the same species pattern as *B. cereus* plus an additional character (excepting *B. thuringiensis*, which had two). The additional characters of these species are, respectively, the fermentation of lactose, greenish-yellow fluorescent pigment, rhizoid growth, pathogenicity for warm-blooded animals, and pathogenicity for insects plus a unique position of the spore in the sporangium. Except for the last-named character, the loss of each of these special features by spontaneous or induced variation has been observed and the resulting cultures could not be differentiated from those of *B. cereus*. Changes in the opposite direction, as indicated by the arrows in figure 1, occurred in two cases; that is, five strains of *B. cereus* were trained to ferment lactose (see

Fermentation Studies) and several strains spontaneously formed a greenish-yellow fluorescent pigment under some conditions of cultivation. It appears, therefore, that under certain circumstances, some of which have been duplicated in the laboratory, these induced variants have evolved from *B. cereus* and that under other conditions they will return to their parental form. The application of the name *B. cereus* to all the strains of this group would provide that the species name of any strain dissociating in any direction (fig. 1) would be unchanged. In other words, both the species pattern of the strain and its species name would remain the same. The designation of the three more stable variants as varieties—var. *mycoides,* var. *anthracis,* and var. *thuringiensis*—has seemed feasible, although not much information was obtained in the case of the last-named variety.

Here again, the International Bacteriological Code of Nomenclature (*16, p. 294*) does not adequately apply. According to it *B. cereus* would be the variant of several species and *B. cereus* and *B. albolactis* would be variants of each other, depending on the direction of the change. The same would be true of *B. cereus* and *B. fluorescens.* To be applicable to this group of organisms, paragraph 6 of the Code might be amended by substituting for the words "from the parent culture" the words "from the pattern of the stable parent species." It would then read, "A variant is an organism showing some variation in some character from the pattern of the stable parent species."

It may not be amiss to state here that before an investigator names a new species he should not only isolate and study a number of strains, but he should also determine the limits of variability of his cultures. In disregarding such a procedure, the worker might discover that a species had lost the special character for which it was named and would discard it as a contamination rather than recognize it as a reversion to the parent species. This is one of the factors that has caused so much confusion in the genus under investigation. Furthermore, inadequate descriptions, as well as the distribution of mislabeled or contaminated cultures, have also played their part in making it almost impossible for the average bacteriologist to identify correctly an aerobic sporeformer.

The taxonomist is naturally limited by his collection. If he does not have enough cultures of a species for study, his knowledge of that species will be incomplete. Five species of group 1—*B. megaterium, B. cereus, B. licheniformis, B. subtilis, B. pumilus*—are fairly well represented in this collection. There are natural varieties or biotypes of each species, and several of these have been dissociated to justify their incorporation in the species. Strains of the remaining groups are found in comparatively small numbers in nature. As they usually grow more slowly and less conspicuously, many of the named strains of these groups were found to have been contaminated and overgrown by *B. subtilis* or some other organism. This collection, therefore, does not contain enough strains to give as clear a taxonomic picture of these groups as is desirable. Nevertheless, the cultures are described and the possible species and subspecies are pointed out, with the acknowledgment that they might have to be revised after the study of a larger collection. Although much has been done, this publication is not intended to be the final word on the genus, the members of which are so often found in all fields of bacteriology.

FACTORS AFFECTING PRODUCTION OF ACETYLMETHYLCARBINOL

Use of Voges-Proskauer Test

The Voges-Proskauer reaction (*174*), demonstrating the presence of acetylmethylcarbinol (acetoin), has long been used to differentiate *Escherichia coli* from *Aerobacter aerogenes*, but its use in the study of the aerobic sporeformers seems to have been limited. Desmots (*44*) noted that *Bacillus mesentericus*, *B. subtilis*, and *Tyrothrix tenuis* produced acetylmethylcarbinol, a finding that was corroborated later by Harden and Norris (*70*). Lemoigne (*97*) studied the decomposition of sugar by *B. subtilis* in more detail. He proved that acetylmethylcarbinol was formed from the sugar in the medium, amounting in some cases to more than 0.3 gram per 100 milliliters of medium containing 4 percent sugar. The quantity reached a maximum and then decreased, even to its complete disappearance. In a later work Lemoigne and others (*98*) tested the distillates from 100 or 1,000 ml. of broth cultures for acetoin and concluded that all species (*B. subtilis*, *B. polymyxa*, *B. anthracis*, *B. cereus*, *B. mycoides*, and *B. megaterium*) were positive. *Bacillus megaterium* formed only 1 to 2 mg. per liter, whereas *B. subtilis* produced more than 1 gm. and *B. polymyxa*, 2.6 gm.

Early in the present work it was noticed that members of the *B. subtilis*-*B. pumilus* group produced acetoin by employing the methods recommended (*150*). The V-P (Voges-Proskauer) test was, therefore, used in the preliminary classification (*145*) as a means of separating these species from the others. Subsequent tests on *B. cereus*, *B. mycoides*, and *B. alvei* showed that some, but not all, strains of these species would also produce acetoin. It was also noticed that the V-P reaction varied with different batches of broth. These observations led to a study of the factors influencing the production of acetoin with a view to standardizing a test that might serve for all cultures. The method described here provides one of the easiest and most reliable tests for separating certain species of the genus *Bacillus*.

Autoclaving the Medium

As uniform results were not obtained with different batches of broth, the first factor studied was the effect of autoclaving. Davis and Rogers (*42*) found that fructose, dextrose, arabinose, and lactose were changed more in a buffer solution than in distilled water and consequently recommended "momentary heating," that is, raising the temperature to 120° C. for 10 minutes and then cooling slowly.

With the above in mind the following test was made: Standard V-P broth[5] was prepared and divided into four parts. One part was tubed and autoclaved at 15 pounds' pressure for 40 minutes (long sterilization), another for 20 minutes (regular sterilization), and one for 12 minutes (short sterilization). The fourth part was sterilized by filtering through a Pasteur-Chamberland bougie, porosity L2, using the technique of Desai (*43*). Five milliliters of the filtrate was pipetted into sterile 18-mm. test tubes and incubated to prove sterility.

Table 1 shows that heating the medium rendered it quite unfit for the formation of acetylmethylcarbinol by *Bacillus cereus*. On the other hand, *B. niger* and *B. aterrimus* were less affected. Despite the good showing of the filtered medium, it was decided that, because this technique might be objectionable and laborious to some workers, other factors should be investigated before recommending its adoption.

TABLE 1.—*Comparison of effect of sterilization of medium by steam and by filtration on production of acetylmethylcarbinol*[1] *in standard Voges-Proskauer broth; 3 days at 37° C.*

Culture	Autoclaved at 15 pounds' pressure for—			Filtered
	40 minutes	20 minutes	12 minutes	
B. cereus:				
No. 201	–	–	±	+++
No. 202	?	?	±	+++
No. 232	–	?	?	++
No. 305	–	±	+	+++
B. aterrimus No. 659	+	+	++	++
B. niger No. 228	++	++	++++	++++

[1] ++++ indicates very strong; +++, strong; ++, weak; +, positive, but very weak; ±, doubtful; ?, very doubtful; –, no reaction.

In the next experiment, the 3 components of the medium were made in triple-strength solutions. Different lots were then prepared by taking aliquots and—

(1) Combining the three and autoclaving (regular medium).
(2) Autoclaving each component separately and combining aseptically.
(3) Combining the buffer and glucose and autoclaving; then adding the autoclaved peptone.

---

[5] Composition: Proteose-peptone, 7 gm.; dipotassium phosphate, 5 gm.; glucose, 5 gm.; and distilled water, 1,000 ml.

(4) Combining the peptone and glucose and autoclaving; then adding the autoclaved buffer.
(5) Combining the peptone and buffer and autoclaving; then adding the autoclaved glucose.
(6) Combining the peptone, buffer, and glucose and sterilizing by filtration.

The results can be briefly summarized. Mediums 1 and 3 were somewhat discolored and proved to be unsuitable for the production of acetoin. On the other hand, Nos. 2, 4, and 5, in which the glucose and buffer were not heated together, were satisfactory and comparable with No. 6, which was sterilized by filtration. This experiment showed, therefore, that heating the medium containing K_2HPO_4 and glucose caused some changes that interfered with the formation of acetoin. This fact was more clearly brought out by autoclaving the medium that had been sterilized by filtration and inoculating with *B. alvei* No. 395 and *B. cereus* No. 232. *Bacillus alvei* failed to show any acetoin in the heated medium, although it produced a 3+ reaction in the filtered control. *Bacillus cereus* formed only a trace in the autoclaved filtered medium and a 3+ in the control.

The question then arose whether the buffer was really necessary. The previous mediums, Nos. 1, 4, and 6, were compared with a similar one containing no buffer (K_2HPO_4). The results showed that *B. subtilis* and *B. cereus* yielded much more acetoin without the buffer. *Bacillus alvei,* on the other hand, did not form it so regularly nor in so great a quantity as it did in the filtered medium or when the buffer was added separately.

At first it appeared as if two mediums would have to be used, one with K_2HPO_4 and one without. In an attempt to obviate this, another experiment was set up to determine whether some other buffer or sodium chloride would fulfill the requirements. For this test, double strength proteose-peptone glucose solution was sterilized and combined aseptically with an equal quantity of autoclaved 1-percent solution of Na_2HPO_4, $(NH_4)_2HPO_4$, or NaCl. In addition, two other batches of peptone glucose broth, one with and one without 0.5 percent NaCl, were made and autoclaved in the usual manner. Some typical results (table 2) show that Na_2HPO_4 gave similar results as K_2HPO_4, as might be expected. whereas $(NH_4)_2HPO_4$ was very poor, even in the case of *B. alvei.* Sodium chloride, however, proved to be as good as potassium or sodium phosphate for this species; it was not harmful to the others and could be incorporated into the medium before sterilization, thus simplifying the procedure.

A large number of cultures were used in comparing this medium with the standard medium and with one without salt. In all cases the modified medium was as good or better, depending on the organism tested. Similar results were obtained with four strains of *Aerobacter aerogenes* from the writers' collection. Banerjea (*5*) and Salmoiraghi (*138*) have also reported better results without the phosphate. Vaughn and others (*172*), however, objected

TABLE 2.—*Effect of buffers and salt on production of acetoin;*[1] *2 to 4 days at 32° C.*

Culture	Proteose-peptone glucose broth,[2] plus—				Proteose-peptone glucose broth	
	K_2HPO_4	Na_2HPO_4	$(NH_4)_2HPO_4$	NaCl	With NaCl[3]	Without NaCl
B. alvei No. 683	+++	++++	[4]−	+++	++++	++
B. cereus No. 201	−	±	−	++++	++++	++++
B. mycoides No. 273	+	+	+	+++	++++	++++
B. subtilis No. 703	++	+++	−	+++	++++	++++

[1] See footnote 1, table 1.

[2] Double-strength broth and 1-percent solutions of the salts were autoclaved separately and combined in equal quantities.

[3] 0.5 percent NaCl added to broth before sterilization.

[4] Some strains of *B. alvei* gave positive results.

to leaving out the buffer because it "is a constituent of the M.R.-V.P. medium most widely used the world over," and Silverman and Werkman (*143*) stated that inorganic phosphate was required. Whatever may be the recommendations for the coliform group, the data presented here show definitely that phosphate added before or after sterilization is detrimental to the production of acetoin by the aerobic sporeforming bacteria. It is, therefore, recommended that an equal quantity of NaCl be substituted for the K_2HPO_4 in the standard medium used for the V-P test.

INCUBATION

Vaughn, Mitchell, and Levine (*170*), working with the coliform group, found that a greater number of cultures produced acetylmethylcarbinol if the temperature of incubation was 30° instead of 37° C. These results were confirmed by Snieszko and Skorka (*149*), who also stated that 28° was satisfactory. As some strains of *Bacillus cereus* (and *B. mycoides*) grow poorly or not at all at 37°, it was necessary to test the effect of incubation temperatures.

Tubes of proteose-peptone glucose solution were incubated at 28°, 32°, and 37° C. for 2, 4, and 6 days. The results (table 3) show that considerable variation appeared in the production of acetoin by *B. cereus* and *B. mycoides*. A temperature of 37° apparently was too high and 28° rather low. By testing a large number of the strains of mesophilic species having a positive V-P reaction, it was found that 32° was suitable for all. In subsequent work, however, it appeared that 37° was slightly more favorable for the *B. subtilis* group and that 45° was optimum for *B. coagulans*.

Although many of the cultures were positive in 2 days, considering the genus as a whole, it was not possible to recommend a single incubation period. There seemed to be no uniformity among strains of a species or between species, either as to rate of formation or disappearance of the acetoin. For this reason several tubes were inoculated and tested at intervals (2, 4, 6, or more days). Slowly developing cultures might require 6 to 20 days. On the other hand, rapidly growing strains needed to be tested early before the acetoin was formed and decomposed.

Table 3.—*Influence of temperature and length of incubation in production of acetoin* [1]

Culture	Temperature at—								
	28° C. for—			32° C. for—			37° C. for—		
	2 days	4 days	6 days	2 days	4 days	6 days	2 days	4 days	6 days
B. cereus:									
No. 248	+	++	++	+	+++	−	+++	+	−
No. 303	++	++	++	++	+++	++	−	−	+
No. 319C [2]	+	+	±	+	+++	++++	+	±	±
No. 402	+	++++	++++	+++	+++	++++	++	++++	+++
B. mycoides:									
No. 273	±	+++	++++	+++	++++	++++	−	−	−
No. 324	±	+++	++	−	++++	+++	−	++++	++++

[1] See footnote 1, table 1.

[2] Dissociant of *B. mycoides* No. 319.

KIND OF PEPTONE

Burton and Rettger (*20*), testing Difco, Witte, Eimer & Amend, and Armour peptones, with and without meat extract, found that meat extract caused a frequent increase in sugar utilization by cultures of the *Aerobacter cloacae-aerogenes* group and recommended a phosphate-meat-extract glucose medium for the V-P test. Dorner and Hellinger (*47*) found the following peptones suitable for acetoin production: Witte's, Difco proteose- and neopeptone, Fairchild's, and Merck's. They were used, however, with a meat-broth base. Georgi and McMaster (*59*) found 7 out of 20 peptones slightly superior. In the recommended methods (*1, 150*) it is stated that Difco proteose-peptone or Witte's peptone should be used. To see if the kind of peptone would influence the production of acetoin in the present study, broths were prepared from Witte's peptone, Bacto-peptone, Bacto-tryptone, and Difco proteose- and neo-peptone, and beef extract. Other brands of peptone were not available.

Bacillus cereus, B. mycoides, and *B. alvei* were consistently positive on proteose- and neo-peptone and were erratic or negative on the other mediums. *Bacillus subtilis, B. niger, B. aterrimus, B. pumilus,* and *B. polymyxa* were positive on Bacto-peptone as well. Tryptone, Witte's peptone, and meat extract were generally inferior. It is recommended, therefore, that proteose- or neopeptone be used for the species of this genus.

Of many other mediums that have been recommended for the V-P test (*20, 70, 75, 97, 98, 99, 100, 123, 178*), only the following were tried in this work: Fumarate broth, sucrose-peptone-salt broth, maltose broth, sucrose bean broth, and 2-percent glucose. Although the fumarate and maltose mediums were unsatisfactory, the sucrose-peptone-salt medium was acceptable, provided the organism utilized that sugar. As many strains of *B. cereus* did not, this medium could not be generally adopted. In an experiment limited to a few strains the sucrose bean broth of Lemoigne and others (*98, 99*) stimulated the production of acetoin by *B. subtilis*. Because the sterile medium gave a faint positive reaction with the creatine reagent, caution had to be exercised in interpreting the results obtained with weakly positive or negative cultures. Two-percent glucose was found to be inferior to the 1-percent generally recommended, thus confirming the results of O'Meara (*123*). Stahly and Werkman (*152*) may have given the explanation of this when they reported that with a rapid fermentation of the glucose the redox potential dropped and glycol accumulated. With the disappearance of the glucose the Eh rose and the glycol was oxidized to acetoin.

REACTION OF MEDIUM

Burton and Rettger (*20*) found that the initial acidity of the medium (containing buffer) had no appreciable effect on the terminal acidity. This was investigated in the present work, using the unbuffered broth as given above at an initial pH of 8.0, 7.0, and 6.0.

The results of the earlier workers were confirmed; namely, that the initial pH had no appreciable effect on the terminal or intermediate acidity. Acetoin formation also was not affected. On the contrary, Vaughn and others (*172*) reported that the pH (6.8 to 7.9) of a heavily buffered medium had a striking effect on the formation of acetoin by the coliform group. Unfortunately, an unbuffered medium was not used as a control.

AERATION

The effect of aeration was considered along with the other factors. O'Meara (*123*) used 5 ml. of broth in ¾-inch test tubes because "increased aeration favors the production of acetoin." Many others have noticed this. Reynolds and Werkman (*131*), for instance, reported that vigorous aeration of cultures of *Escherichia coli* resulted in a positive reaction, and Mickelson and Werkman (*113*) observed that aeration under pressure increased the production of acetoin by *Aerobacter indologenes*. Owing to the difficulties of keeping cultures aerated under pressure, different degrees of aeration were obtained in the present work by putting

5 ml. of the modified broth into test tubes of different diameters as follows:

Small tubes, diameter 15 mm.; depth of liquid approximately 35 mm.
Regular tubes, diameter 18 mm.; depth of liquid approximately 25 mm.
Large tubes, diameter 22 mm.; depth of liquid approximately 18 mm.

A few tests were made using a more shallow layer in 250-ml. Erlenmeyer flasks; but, as these gave about the same results as the large tubes and the flasks were awkward to handle, they were not used to any extent.

Before adding the reagents for the V-P reaction, the broth culture was poured into medium-size tubes to avoid any effect that the size of the tubes might have upon the development of the color. In every case, using many strains of *Bacillus cereus* and *B. subtilis,* the cultures incubated in the largest tubes gave the strongest reaction for acetoin. Furthermore, with the large tubes a positive reaction was obtained in a shorter incubation period than was possible with the smaller tubes. The cultures in the smallest tubes (15 mm.) were decidedly poor. Certain strains (*B. mycoides* No. 936 and *B. aterrimus* No. 740) that were definitely positive in the large tubes were either negative or doubtful in the small tubes. On the other hand, *B. polymyxa* and *B. alvei* produced acetoin equally as well in the small as in the larger tubes, although usually not so rapidly.

TEST REAGENTS FOR ACETYLMETHYLCARBINOL

Attempts too numerous to mention have been made to increase the delicacy or hasten the V-P reaction, but judging by the literature no method has been accepted above all others. It was only natural, therefore, that a few of the more common methods should have been compared in this work. In the first tests, the KOH-copper sulfate-ammonia method (*150*) was used and gave good results. Later this method was compared with the alpha-naphthol method of Barritt (*6*) and the creatine method of O'Meara (*123*). Previously, Vaughn, Mitchell, and Levine (*171*) had compared the KOH, the alpha-naphthol, and the creatine methods and strongly recommended the alpha-naphthol. They modified O'Meara's reagent by dissolving 0.3 percent creatine in the KOH before using, whereas O'Meara added the dry powder to the test solution.

These two creatine methods (*123, 171*) were thoroughly tested on the cultures under observation. When fresh, the 0.3 percent creatine in NaOH gave as good results as the dry powder, but after aging a few days it became weaker. After standing a month at room temperature its strength was reduced so much that a 1+ or a plus-minus reaction was obtained in contrast to a 4+ reaction by O'Meara's method. In a laboratory where numerous tests are made each month, the solution of creatine might be more economical than the dry powder, as pointed out by Vaughn and others

(*172*). On the other hand, when only a few tests are made, 0.5 to 1 mg. of the powder added to each tube would be much more reliable and would use less creatine.

The alpha-naphthol method was more difficult to read than O'Meara's or the copper sulfate methods, contrary to the findings of Barritt (*7*). After the completion of this work, Batty-Smith (*8*) gave a good review of the literature on the detection of acetoin and, on the basis of his tests with *Aerobacter aerogenes,* recommended a combination of the alpha-naphthol, creatine, and ferric chloride reagents. Incidentally, he used a medium containing a phosphate and an incubation temperature of 37° C. for 2 days, relying on the sensitivity of the method rather than on the improvement of growth conditions for the maximum production of acetoin by the organism. DeGraaf (*69*), Coblentz (*33*), Stotz and Raborg (*160*), and Salmoiraghi (*139*) have also suggested modifications in the methods for the detection of acetoin.

In the writers' laboratory the method of O'Meara was entirely satisfactory: An equal volume of 40 percent NaOH was added to the culture and 0.5 to 1.0 mg. of creatine was dropped on the surface from a knife point. The tube was shaken and observed within 30 minutes to an hour. As the broth did not contain a phosphate, its color was very slight and there was no interference with the bright red color shown in the positive V-P reaction.

Conclusions on Production of Acetylmethylcarbinol

From the results obtained it was concluded that for the production of acetylmethylcarbinol the cultures of the genus *Bacillus* should be grown in a broth containing 7 gm. of proteose- or neopeptone, 5 gm. glucose, and 5 gm. NaCl per liter. The medium should be dispensed 5 ml. per regular test tube (18 mm. in diameter), incubated for 2, 4, and 6 days at 32° or 37° C. for the mesophilis and 45° for the thermophilic cultures, and the V-P test made by the creatine or a similar sensitive method. Longer periods of incubation, 10 to 20 days, might be necessary in a few instances.

The formation of acetylmethylcarbinol proved to be one of the most reliable characters of this group and was very useful in the separation of several species.

STORAGE OF FAT

In Smith's and Clark's preliminary classification of this group (*145*), the occurrence of fat globules in the rods grown on glucose-nutrient agar was used to separate *Bacillus megaterium* and *B. cereus* from the other species. After 3 or 4 days' incubation at 28° C. the cells showed large fat globules when stained in a wet mount with Sudan III (saturated alcoholic solution). The globules were pink or light orange and were not in so sharp a contrast with the rest of the cell as was desired. Nile-blue sulfate (*37, p. 80*) and oil red O (*37, p. 44*) were tried, but these stains were found to be inferior to Sudan III. Sudan IV gave slightly better results, because less precipitate was formed in the wet mount.

Hartman in 1940 (*71*) introduced Sudan black B as a stain of bacterial fat. It had been used previously by Lison (*105*) to stain fat in tissues, because it proved superior to a large number of fat stains, including Sudan III, and was specific for fat. Hartman recommended a saturated solution of Sudan black B in ethylene glycol, because there was no evaporation from the wet mount, consequently no streaming, and the mount might be examined at one's leisure. The fat droplets were stained dark blue or black and stood out in good contrast.

With Hartman's method all the members of this genus were found to store demonstrable fat. This has also been observed by Imšenecki (*77*), using Sudan III. Most strains of *B. megaterium* and *B. cereus* formed numerous large globules; others formed fewer and smaller ones. In strains of *B. megaterium* containing only small droplets it was very hard to decide with Sudan III whether they were fat-positive or fat-negative. There was no doubt, however, that they were positive, when Hartman's method was used. *Bacillus brevis,* which had been suspected of being fat-positive when stained with Sudan III, was clearly positive with Sudan black B. Rods of *B. subtilis, B. aterrimus, B. niger, B. firmus,* members of the *B. circulans* complex, and those of the round-spored group contained a few small globules that could be seen easily with the black B stain but were not visible with the Sudan III.

Burdon and others (*18*) recommended making a suspension of the organisms in 0.5 ml. of a saturated alcoholic solution of Sudan black B, spreading a drop on a slide, drying, and counterstaining with safranin. They state, "even the most minute fat droplets are brilliantly revealed." In the fat-negative group they place *B. mesentericus* (probably the American strain that is *B. pumilus*), *B. subtilis* (Ford strain), *B. subtilis* (Marburg strain), and *B. vulgatus.* In a later publication Burdon (*17*) lists these species among those in which fatty material is usually present.

The production of fat by the genus is, therefore, quantitative rather than qualitative. This applies not only to different species but also to different strains within a species. Obviously this character must be used with discretion and not as a definite criterion on which to separate certain species from others.

In place of the presence of fat the writers selected a criterion used by Gibson and Topping (*64*) to differentiate *B. megaterium* and *B. cereus* from the rest of the bacilli. The normal rods of these two species cultivated on glucose- or glycerol-nutrient agar appeared vacuolated or foamy when smeared and stained with a dilute solution of fuchsin, largely because of the many large fat droplets. In the case of a variant the presence of many large swollen unstained forms (shadow-forms) was equally instructive.

The smaller rods of the other species colored evenly with fuchsin because the fat globules were fewer and much smaller. Shadow-forms were relatively scarce and much smaller. There seemed to be less difficulty in interpreting the dilute fuchsin stain, and it is, therefore, recommended for general use.

AGGLUTINATION RESPONSES

The serology of the members of the genus *Bacillus* was investigated to a very limited extent, because facilities for such work were available to one of the writers (F. E. C.) for only a short time. Young healthy rabbits, in successive lots of 3 to 5 animals each, were immunized against the whole cultures selected as antigens through sublethal injections of these cultures. Eight to ten injections, varying from 1.0 ml. of nephelometer turbidity 2 to 3.0 ml. of nephelometer turbidity 3 of phenolized antigen (0.3 percent), given at approximately half-weekly intervals, produced satisfactory serum titers. Agglutination tests were performed with young whole cultures harvested from nutrient agar surfaces.

The agglutination responses of seven strains of *B. alvei*, four strains of *B. circulans*, and five of *B. laterosporus* to antiserums for two strains of *B. alvei* and one of *B. laterosporus* are given in table 4. The three antiserums agglutinated all the strains of their homologous species with one exception, No. 686. There was no cross agglutination among species. *Bacillus circulans* did not cross-agglutinate with either of the *B. alvei* antiserums, although these species are closely related.

Table 4.—*Serologic agglutination responses of Bacillus alvei, B. circulans, and B. laterosporus*

Agglutinogens	Agglutinins[1]		
	B. alvei		*B. laterosporus* No. 681
	No. 662	No. 685	
B. alvei:			
No. 662	320	160	0
No. 683	160	160	0
No. 684	640	320	0
No. 685	320	640	0
No. 686	–	320	0
No. K[2]	320	320	0
No. M[2]	320	160	0
B. circulans:			
No. 676	0	0	0
No. 677	0	0	0
No. 678	0	0	0
No. 679	0	0	0
B. laterosporus:			
No. 314	--------	--------	1,280
No. 340	--------	--------	640
No. 661	--------	--------	80
No. 681	0	0	640
No. 686	--------	--------	640

[1] Reciprocal of highest serum dilution giving positive agglutination.

[2] Discarded after these tests.

Antiserums were developed against *B. subtilis* No. 231, *B. vulgatus* No. 238, *B. aterrimus* No. 230, and *B. niger* No. 254. Agglutination responses by a number of strains of the *B. subtilis* group to the four antiserums are presented in table 5. The results offer no basis for the separation of *B. subtilis, B. vulgatus, B. aterrimus,* and *B. niger.* The responses as a whole were erratic and tended to be strain-specific, with cross agglutination occurring only at the lower titers. The results are in accord with those presented below, which show that *B. vulgatus* is in synonymy with *B. subtilis* and that *B. aterrimus* and *B. niger* are black-pigmented varieties of *B. subtilis.*

TABLE 5.—*Serologic agglutination responses of Bacillus subtilis, B. vulgatus, B. aterrimus, and B. niger*

Agglutinogens	Agglutinins[1]			
	B. subtilis No. 231	*B. vulgatus* No. 238	*B. aterrimus* No. 230	*B. niger* No. 254
B. subtilis:				
No. 231	10,240	80	80	80
No. 237	320	640	160	160
No. 242	0	0	640	0
No. 330	40	10,240	80	160
B. vulgatus:				
No. 238	40	10,240	80	80
B. aterrimus:				
No. 230	0	0	10,240	40
No. 259	80	80	10,240	0
No. 260	160	80	10,240	40
No. 261	160	80	5,120	40
No. 262	5,120	80	160	80
No. 274	0	0	2,560	0
No. 275	80	0	5,120	0
No. 276	40	40	2,560	0
B. niger:				
No. 219	160	80	0	320
No. 220	40	40	40	2,560
No. 223	160	160	0	320
No. 224	80	320	80	1,280
No. 225	160	80	80	640
No. 254	0	40	0	2,560
No. 265	320	320	160	640

[1] Reciprocal of highest serum dilution giving positive agglutination.

Fourteen antiserums were prepared against strains of *B. brevis.* Although satisfactory homologous titers were given by 7 antiserums (*B. brevis* Nos. 604, 605, 616, 628, 632, 641, and 664), cross-agglutination responses were not observed. The 7 remaining antiserums (*B. brevis* Nos. 603, 611, 612, 614, 622, 635, and 640) were effective against 2 or 3 strains (table 6). Owing to the specificity of the antiserums, the agglutination reactions of *B. brevis* were taxonomically unsatisfactory.

TABLE 6.—*Serologic agglutination responses of Bacillus brevis and B. laterosporus*

Agglutinogens	Agglutinins[1]								
	B. brevis							*B. laterosporus*	
	No. 603	No. 611	No. 612	No. 614	No. 622	No. 635	No. 640	No. 314	No. 661
B. brevis:									
No. 603	5,120	0	0	0	0	5,120	0	0	0
No. 611	0	5,120	0	0	0	0	20,480	0	0
No. 612	0	0	20,480	20,480	20,480	0	0	0	0
No. 614	0	0	20,480	20,480	20,480	0	0	0	0
No. 622	0	0	20,480	20,480	20,480	0	0	0	0
No. 635	5,120	0	0	0	0	5,120	0	0	0
No. 640	0	2,560	0	0	0	0	20,480	0	0
B. laterosporus:									
No. 314	0	0	0	0	0	0	0	5,120	10,240
No. 661	0	0	0	0	0	0	0	5,120	10,240

[1] Reciprocal of highest serum dilutions giving positive agglutination.

The two strains of *B. laterosporus* also included in table 6 did not react with any of the *B. brevis* antiserums in spite of the close relationship of the two species.

Antiserums effective against one or more strains of *B. brevis* failed to agglutinate cultures of *B. megaterium, B. cereus, B. subtilis, B. niger, B. aterrimus, B. pumilus, B. alvei, B. polymyxa,* and *B. macerans.*

To determine whether the homologous and heterologous agglutinogens within a cross-agglutination group showed similar or dissimilar absorption capacity, serum against *B. brevis* No. 622 was treated with an excess of the following whole-culture antigens: *B. brevis* Nos. 612, 614, 622, and 632, the latter antigen having failed to cross-agglutinate. The unabsorbed serum had an agglutinin titer of 20,480 for the first three cultures (table 6) and a negative one for strain No. 632. The antiserum prepared with culture No. 622 and exposed to excessively turbid cell suspensions of strain No. 612 for 30 minutes gave positive agglutination of cultures Nos. 612, 614, and 622 at dilutions of 1 to 20 and 1 to 200, and negative agglutination at 1 to 2,000 and 1 to 20,000. After the second exposure, positive agglutination at 1-to-10 dilution was observed; and after a third exposure, no agglutination. Similar titer reductions were shown after absorption with either culture No. 614 or culture No. 622. The serum against *B. brevis* No. 622 exposed to *B. brevis* No. 632 still retained a titer of 20,480 for cultures Nos. 612, 614, and 622.

The usefulness of serology in the taxonomy of this genus is uncertain, especially since McCoy's able demonstration (*108*) of positive and negative responses among descendants of a single culture. Some serological studies reported in the literature are of little value because of the prevalence of misnamed strains and the confusion of identities that have existed between the American and European *B. mesentericus* and between *B. cereus* and *B. subtilis.*

Several workers using vegetative cells as antigens recorded a few responses, apparently between strains of separate species. Carbone and others (*22*) studied the agglutination of 26 strains of a number of species by 13 antiserums prepared with bacilli of the *B. mesentericus* group. Although they concluded that most of the species were antigenically distinct, they regarded the 4 species *B. aterrimus, B. mesentericus fuscus, B. tyrosinogenes,* and *B. carotarum* as a subgroup because of their co-agglutination at high titer. Their *B. mesentericus fuscus* was probably the European *B. mesentericus,* which will be shown later to be the same as *B. subtilis.* Also closely related to *B. subtilis* are *B. aterrimus* and *B. tyrosinogenes.* Carbone's responses for the first 3 species, therefore, were probably within the same species. In the case of *B. carotarum,* however, 2 distinct species would seem to be antigenically similar, as the cultures of *B. carotarum* in the writers' collection proved to be identical with *B. megaterium.* Sievers and Zetterberg (*142*) observed positive complement fixation between strains of the following separate species: *B. mycoides* antiserum with *B. vulgatus* (*B. subtilis*); *B. cereus* antiserum with *B. vulgatus; B. subtilis* antiserum with *B. cereus* and *B. mycoides;* and *B. vulgatus* antiserum with *B. mycoides. Bacillus cereus* antiserum also reacted with *B. subtilis* by the precipitin test.

The agglutination reactions of *B. macerans* and *B. polymyxa* were investigated by Porter, McCleskey, and Levine (*126*). All 16 strains of *B. macerans* were agglutinated by the 2 homologous serums tested. Although each of 9 *B. polymyxa* serums reacted with its specific strain and a few others, none agglutinated all 71 cultures of *B. polymyxa.* There was no reaction between the serum of one species and the cells of the other.

Lamanna (*89, 90, 91*), seeking to avoid the complex antigen of the vegetative cell, used the serological reaction of the spores and separated *B. subtilis* from *B. vulgatus* by precipitogens obtained from spores. *Bacillus vulgatus* has often been called the Ford strain of *B. subtilis,* and, more recently, *B. licheniformis* (*62, 63*). Lamanna also found that the spores of 10 strains of *B. cereus* and 1 of *B. mycoides* were agglutinated at low titer by the spore antiserum of *B. agri* (probably *B. brevis*). His remaining antiserums, with the exception of the spore antiserum of *B. cereus,* are believed to be too specific for identifying a well-represented species. The agglutination of the spores of 31 cultures of *B. cereus,* including several strains of *B. mycoides,* by the spore antiserum of a strain of *B. cereus,* however, was especially noteworthy. It is regrettable that this antiserum was not tested against more strains of the *B. cereus* group, for it gave promise of being very useful.

The results of the serological studies of the present work, though meager, point to a similarity between the serology and bacteriophagy of these organisms. It was not unusual to find that a bacteriophage developed against a strain of a particular species lysed only that one strain, or that a second bacteriophage for another culture of the same species dissolved several strains, or that a third bacteriophage prepared with still another culture was active against all the strains of the particular species.

The antiserums of the few bacilli studied by the writers resembled the bacteriophages in their reactions with the strains of each homologous species. The agglutinins for *B. brevis* No. 604, for example, reacted only with culture No. 604; serum against *B. subtilis* agglutinated part but not all of the cultures of *B. subtilis;* and the antiserum of *B. alvei* No. 685 agglutinated all the strains of *B. alvei.* Although this apparent similarity between the bacteriophagy and serology of the bacilli may or may not have a common basis, the possibility should not be overlooked.

BACTERIOPHAGY

Bacteriophage was used for the separation of four species during the first part of this study, when knowledge of the cultures was meager and the reliability of most reactions uncertain. As the work progressed other reactions were found that were as dependable as the bacteriophage and much simpler in application. Although sensitivity or resistance to a strain of bacteriophage has not been included in the final definition of any of the species, the methods and results of the experiments are given here for those interested in bacteriophage as a taxonomic tool.

Each strain of bacteriophage was isolated by the following technique: About 10 gm. of garden soil was added to 100 ml. of a 16-hr. nutrient broth culture of the organism against which a bacteriophage was desired. After incubating for 2 or 3 days at room temperature the mixture was filtered through paper and then through an L2 Pasteur-Chamberland filter. Ten milliliters of an 8-hr. culture of the same organism was added to the filtrate and held at 28° C. overnight. Ordinarily it was necessary to repeat the filtration and the addition of a young culture only once or twice before the reinoculated filtrate appeared clear and limpid after its overnight incubation.

Upon the first evidence of bacteriophage, a few drops of the lytic culture was thoroughly mixed with 5 ml. of a 6- to 8-hr. culture of the sensitive bacillus and 2 to 3 drops of the mixture was spread over the surface of an agar plate. If bacteriophage was present, the resulting growth was punctured with lysed areas, or plaques. A small inoculating loop was touched to a single well-isolated plaque and then dipped into a tube of another young broth culture. When this culture was dissolved, the quantity and the potency of the lytic agent were increased by combining it daily with larger and larger numbers of the susceptible cells. If the lysed culture became cloudy with secondary growth it was filtered before any further addition of the sensitive organism. The strength of the bacteriophage was roughly determined by serial dilution in tubes containing 9 ml. of sterile nutrient broth. One milliliter of a 16-hr. culture of the bacillus against which the bacteriophage had been developed was pipetted into each of the dilution tubes. If clearing was observed in 24 hr. in the 10^{-7} or 10^{-8} dilution, the lytic filtrate was considered potent enough for testing unknown strains.

Susceptibility to a bacteriophage was determined by inoculating two tubes of broth with a drop of a 24-hr. culture; 1 ml. of the active filtrate was then added to one tube; the other tube served as the control. The tubes were held at room temperature and observed at 4, 8, 24, and, occasionally, 48 hr. A clear bacteriophage tube with a turbid control was considered evidence of lysis. For additional information a loopful from each tube was streaked on a nutrient agar slope. Lack of growth or unmistakable plaques on the slope inoculated from the phage tube was regarded as proof that the organism was sensitive to the bacteriophage. The results were checked at least once by repeating the procedures.

Not all strains of bacteriophage could be used taxonomically, because too many were specific for one or a few cultures of a species. A lytic agent developed against *Bacillus megaterium* No. 240 and one against No. 258 proved to be too restricted in their actions to aid in the identification of the species (table 7). The effects of these two bacteriophages were identical and had no relation to the pigment of the cultures, to their stage of growth, or to any other property so far as could be determined. The bacteriophage developed against No. 239, on the other hand, lysed

TABLE 7.—*Specificity of strains of bacteriophage for Bacillus megaterium*

Cultures of *B. megaterium*	Type of growth	Lysis by bacteriophage for culture—		
		No. 240	No. 258	No. 239
No. 234	White, smooth	−	−	+
No. 239	Yellow, smooth	−	−	+
No. 240	White, smooth	+	+	+
No. 241	Cream, smooth	−	−	+
No. 245	White, smooth	+	+	+
No. 257 [2]	White, intermediate	+	+	+
No. 258 [2]	Yellow, rough	+	+	+
No. 268	Cream, rough	−	−	+
No. 269	Cream, smooth	+	+	+
No. 270	Yellow, rough	−	−	+
No. 271	Yellow, intermediate	−	−	+
No. 283	White, smooth	+	+	+
No. 284	do	+	+	+
No. 285	do	−	−	+

[1] + indicates lysis; −, no visible lysis.

[2] Rough stages of No. 234.

all the strains included in the table and 83 percent of all the strains of *B. megaterium* studied.

Too great a specificity was also shown by the three bacteriophages for *B. laterosporus,* and none capable of attacking all the strains of this species was isolated (table 8). Again, no other difference was recognizable between the susceptible and the resistant strains.

Table 8.—*Specificity of strains of bacteriophage for Bacillus laterosporus*

Cultures of *B. laterosporus*	Lysis by bacteriophage[1] for culture—		
	No. 661	No. 314	No. 347
No. 314	−	+	+
No. 340	−	+	+
No. 347	+	+	+
No. 590	−	+	+
No. 661	+	−	−
No. 681	+	−	−
No. 682	+	−	−

[1] + indicates lysis; −, no visible lysis.

For the isolation of a bacteriophage for *B. cereus,* the choice of strain No. 201 was especially fortunate. The resulting lytic agent attacked all strains of *B. cereus,* those of the closely related anthrax bacillus, and the nonrhizoid dissociants of *B. mycoides.* The rhizoid stage of *B. mycoides,* however, was not lysed.

A bacteriophage against *B. mycoides* No. 319 was developed that lysed the rhizoid strains, their nonrhizoid variants, and cultures of *B. cereus.* Because it was isolated some time after the bacteriophage for *B. cereus* No. 201, it was not used so extensively as the latter.

A lysogenic agent was also developed that attacked the smooth stage of *B. subtilis* No. 231. At first it lysed only that particular form, but after its potency had been increased it dissolved the rough stage as well. When other strains of *B. subtilis* were tested with this bacteriophage, the smooth ones and a few of the rough ones were susceptible. If a rough, resistant *B. subtilis* was dissociated, the smooth variant was lysed. This bacteriophage, however, was not used to study all of the strains available—the volume of work required for the dissociation of the rough strains was prohibitive.

Two other bacteriophages, one for *B. pumilus* No. 236 and one for *B. brevis* No. 605, attacked a high percentage of the cultures of their respective species (table 9).

The findings of Francis and Rippon (*54*) in their study of 4 strains of bacteriophages (types A, B, C, and D) for *B. polymyxa* agree closely with those for other species described here. Their

TABLE 9.—*Effectiveness of bacteriophagy in the identification of certain species*

Species	Strains	Lysis by bacteriophage for—				
		B. megaterium No. 239	*B. cereus* No. 201	*B. pumilus* No. 236	*B. polymyxa* (phage C)	*B. brevis* No. 605
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
B. megaterium	90	83				
B. cereus	145		[1]100			
B. pumilus	70			100		
B. polymyxa	25				72	
B. brevis	51					88

[1] *Bacillus mycoides* was resistant, but all its nonrhizoid dissociants were lysed.

39 strains of *B. polymyxa* exhibited variable susceptibility, ranging from 41 percent lysis by type A to 100 percent by type C. Type C—kindly supplied by Francis and Rippon—attacked only 72 percent of the strains of *B. polymyxa* in the writers' collection, however. It is regretted that time did not permit further study of the resistant strains, for it seems entirely reasonable that they were in the rough stage and that their smooth dissociants would have been susceptible.

Each of the five bacteriophages listed in table 9 was tested against several strains of the most closely related species. Cultures of *B. circulans* and *B. macerans*, for example, were examined for sensitivity to bacteriophage type C for *B. polymyxa*. None of the strains selected gave any indication of cross reactions among species.

STUDIES ON DISSOCIATION

Variability of bacteria has for some time been an accepted phenomenon in microbiology. In the laboratory these variations may be spontaneous or they may be induced by specific treatments. Their counterparts may often be isolated from natural materials, thereby lessening the artificiality of the changes brought about in the laboratory. Some of the variations, such as the acquisition and loss of the ability to attack a specific carbohydrate, appear to be easily reversible. (See Section on Fermentation Studies.) Other modifications are much more stable, and reversion has often been impossible.

Any study on taxonomy would naturally include a consideration of the variability encountered within a species, and the ideal classification would allow for this in the characterization of each species. Because the amount of work required would be enormous, only a small beginning could be made in this investigation. Several strains heretofore given species rank were found to be separated from definite species by only one character. The dissociation of these strains, resulting in the loss of the differentiating property, provided grounds for reducing them to the status of varieties. Consequently some species of long standing have been eliminated.

Dissociation of Bacillus mycoides

HISTORICAL BACKGROUND

Bacillus mycoides Flügge is widely known and easily recognized because of its unique colony form. In 1881 Koch (*85*) was undoubtedly referring to *B. mycoides* when he mentioned an unnamed bacillus, very resistant to heat, whose colonies were like interwoven mattings of roots. Five years later, Flügge (*51, p. 324*) named and described *B. mycoides* as a sporulating rod resembling the anthrax bacillus in size, with oval central spores and colonies of tangled, rootlike, ramifying threads.

Variation in the colonial form of *B. mycoides* was first reported by Nadson and Adamovic in 1910 (*117*), although their observation that the unusual colony resembled an actinomycete was not substantiated by later workers. In 1927 Nyberg (*120*) found that five of six cultures of *B. mycoides* when transferred in broth or peptone water for several months showed some typical rhizoid colonies and also some of a very different appearance. He stated that these atypical colonies closely resembled those of *B. mesentericus.* Later (*121*) he continued his earlier work with a larger number of cultures of *B. mycoides* and their variants and was not able to separate them physiologically from cultures of *B. mesentericus* and *B. subtilis.* In this connection it should be considered that *B. cereus* was often called *B. subtilis* at that time and that it is possible Nyberg made the same error. Perlberger (*125*), Pringsheim and Langer (*129*), Oesterle and Stahl (*122*), Stapp and Zycha (*154*), Brunstetter and Magoon (*15*), Lewis (*102*), Lewis and Worley (*104*), Gause (*57*), and Rautenstein (*130*) also observed a change in the colonial form of *B. mycoides.*

In 1931 Dooren de Jong (*45*) described a sporeforming bacillus and named it *B. undulatus.* The two cultures bearing this name, which the writers received from Dooren de Jong through Porter, were *B. cereus* and agreed with the original description of *B. undulatus.* In 1933 Dooren de Jong (*46*) also observed the nonrhizoid colonies of *B. mycoides* and stated that these smooth atypical forms were identical with *B. undulatus.* Soriano and Soriano (*151*) recognized that the atypical strains of *B. mycoides* were in fact *B. cereus,* described by Frankland and Frankland in 1887 (*55*). Taxonomic studies of these two species reported here led to the same conclusion.

DISSOCIANTS OBTAINED

In the writers' collection were 21 cultures named *Bacillus mycoides* and 1 named *B. praussnitzii,* which is said to differ from *B. mycoides* only in its ability to ferment lactose (*93, p. 495*). These strains were cultivated on nutrient agar from 3 to 20 years, and during that time they gave no indication of departing from their characteristic colonial form. After preliminary observations had shown that variation might occur in a fairly large volume of broth, a colony of each of the 22 rhizoid cultures was picked from a nutrient agar plate into a flask containing 100 ml. of nutrient broth. These cultures were held at 28° C. and plates of nutrient agar were made between the third and tenth day. In most cases the plates showed varying numbers of dense, soft, nonadherent colonies mixed with the typical mycoid ones (pl. 1, *A*). A few of the cultures dissociated more slowly, and nonrhizoid colonies did not appear until after an incubation of 3 to 4 weeks. Dissociation was apparently hastened by the use of small inoculums.

The variant colonies had the characteristic appearance of those of *B. cereus,* owing to the adherence of the cells in long parallel chains, described by different writers as resembling ground glass, galvanized iron, or watered silk. The chains did not twist together in threads, as in the rhizoid colony, but were spread out in a loose, wavy arrangement as though they had been combed. It was impossible to distinguish between the variant colonies of *B. mycoides* and the colonies of *B. cereus.*

In one experiment daily serial transfers of *B. mycoides* to tubes containing 5 ml. of broth caused colony variation in 8 to 10 days, whereas cultures grown without transfer in single tubes of broth did not dissociate until after 4 to 6 weeks. Cultures inoculated into beef broth diluted to only 10 percent of its normal nutrients also dissociated, but not so quickly as in a richer broth. On the other hand, cultures transferred serially 50 times on nutrient agar did not lose their characteristic rhizoid growth, although 2 out of 5 cultures dissociated on glucose-nutrient agar after 30 serial transfers. The rhizoid form did not, therefore, always remain stable when cultivated on a solid medium, but dissociation was difficult and infrequent compared with that obtained in a large volume of broth.

VITAMIN REQUIREMENTS

The work of Robbins and Ma (*133*) on the growth requirements of a strain of *Fusarium avenaceum* suggested that a lack of nutritional elements in broth as compared with agar might be a factor in the dissociation of *Bacillus mycoides.* Like the *Fusarium* culture, this organism failed to grow in a solution of mineral salts and glucose but grew on the same solution solidified or semisolidified with agar. As it was the biotin in the agar that was effective with the *Fusarium* culture, the following growth factors were used for the growth of *B. mycoides:* Biotin, riboflavin, thiamine hydrochloride, nicotinic acid, and calcium pantothenate. Each vitamin was tested separately by adding 0.1µg., 1µg., 5µg., and 10µg. to a tube of glucose basal solution. *Bacillus mycoides* grew and formed acid from glucose when 1µg. or more of biotin was added to a tube of medium. It failed, however, to grow with any of the other vitamins in the concentrations used. This indicated that the growth-promoting substance in agar for *B. mycoides* was biotin.

It was assumed that some biotin was present in nutrient broth, because good growth occurred even when the broth was diluted. Nevertheless, it was thought advisable to test the effect of the addition of biotin to diluted broth. One microgram of biotin in sterile solution was added to each tube of nutrient broth, which had been diluted to 10 and to 25 percent of its original strength. The cultures of *B. mycoides* inoculated into this biotin medium were examined at 5 to 7 days. Variant colonies were found on the plates of most cultures at 5 days and on the plates of all cultures at 7 days. These results were comparable with those obtained above without added biotin. The quantity of biotin, therefore, had no effect on dissociation.

EXAMINATION OF DISSOCIANTS

Microscopically the cells of both *Bacillus mycoides* and *B. cereus* were large rods, 0.9μ to 1.2μ wide, with squared ends and oval centrally placed spores that swelled the sporangium only slightly if at all. They normally contained a large number of vacuoles, which gave the lightly stained cells a foamy appearance. Generally the rods of *B. mycoides* were somewhat longer and a trifle thinner than those of *B. cereus;* they formed longer chains, and did not sporulate so profusely. Although many of the cultures of *B. cereus* in this collection formed spores quickly and abundantly, the extent of sporulation varied with the strain. The cells of the dissociants of *B. mycoides* when first isolated generally resembled those of the rhizoid stage in size and formed no spores. With continued transfer on agar they became shorter and thicker, appeared singly or in short chains, and slowly resumed sporulation. Three years after the separation of the nonmycoid cultures of *B. mycoides* a comparison of the numbers of spores seen in cultures of these dissociants and of *B. cereus* was made (table 10). The

TABLE 10.—*Comparison of numbers of spores formed by cultures of Bacillus cereus and by the nonrhizoid dissociants of B. mycoides*

B. cereus	Spores [1] in—		Dissociants of *B. mycoides* [2]	Spores [1] in—	
	24 hours	48 hours		24 hours	48 hours
No. 410	+++	++++	No. 233 (C)	+	+
No. 538	+	+	No. 273 (C)	++++	++++
No. 539	+	++	No. 273 (16)	+	++++
No. 540	+	+	No. 306 (C)	++++	++++
No. 542	++	++++	No. 306 (18)	+	+
No. 543	++	++	No. 317 (C)	++	++++
No. 544	+	+++	No. 317 (4)	+	++
No. 545	++	+++	No. 317 (16)	++++	++++
No. 589	+	++	No. 318 (C)	+	+
No. 599	++++	++++	No. 319 (C)	+++	+++

[1] + indicates an occasional spore; ++, few spores; +++, numerous spores; ++++, spores in the majority of the rods.

[2] The designations in parentheses identify the various dissociants.

data show that *B. cereus* and the variants of *B. mycoides* cannot be separated on the basis of sporulation. Strain No. 317 illustrates that dissociants of the same rhizoid culture of *B. mycoides* may not form like numbers of spores. A large number of physiological tests on *B. mycoides,* on its dissociants, and on *B. cereus* failed to show any consistent difference that might be used for separating them.

Knight and Proom (*84*) found that the nutritional requirements of *B. cereus* and *B. mycoides* were the same. They observed the dissociation of the rhizoid strains and supplied some interesting information on the temperature relationships. They reported that strains of *B. mycoides* grew poorly or not at all at 37° C., while those of *B. cereus* showed a slight preference for 37° over 28°. They dissociated a culture of *B. mycoides* that would not grow at 37° and found that the nonrhizoid isolate grew slightly better at 37° than at 28°.

LYSIS OF DISSOCIANTS BY BACTERIOPHAGE

A pure-line bacteriophage against *Bacillus cereus* No. 201 was isolated from soil. (See section on Bacteriophagy.) It lysed all strains of *B. cereus* in the collection, but it was inactive against the rhizoid form of *B. mycoides* and *B. praussnitzii.* After dissociation, however, the variants of these two species were readily dissolved by this phage. Another bacteriophage developed against *B. mycoides* No. 319 did lyse the rhizoid forms; it also dissolved their dissociants and cultures of *B. cereus.*

STABILITY OF THE DISSOCIANTS

The nonrhizoid stage of *Bacillus mycoides* was very stable, and only 1 of the 33 variant cultures selected for study returned spontaneously to the rhizoid form. It may not have been fully dissociated; it reverted very soon after its separation. The remaining 32 cultures were carried on agar along with the stock cultures for 9 years, plated from time to time, and carefully examined for rhizoid colonies. None were ever found.

In order to stimulate reversion, cultures of the dissociants of *B. mycoides* and also some cultures of *B. cereus* were grown on different mediums under varying conditions of pH, oxygen tension, temperature, humidity, and surface tension and in immune serums and bacteriophage. No mycoid colonies were ever observed, although the cultures often became very rough and aborescent. Reversion should be possible, but the conditions to bring it about have not been discovered.

Dissociation of Bacillus praussnitzii

As reported previously (*93, p. 495*), *Bacillus praussnitzii* was found to resemble *B. mycoides* except for its active fermentation of lactose. The writers observed that it dissociated in the same way as *B. mycoides* and that its variants also fermented lactose. These could not be differentiated from the so-called *B. albolactis,* which in all other respects was identical with *B. cereus*. As the utilization of lactose is an uncertain property (see section on Fermentation Studies), it is recommended that these lactose-positive strains be considered as biotypes rather than as varieties.

DISSOCIATION OF BACILLUS ATERRIMUS

The similarity among *Bacillus subtilis* (*B. vulgatus*), *B. aterrimus,* and *B. niger* was noted early in this work. Clark and Smith (*31*) reported that *B. aterrimus* formed a black pigment only upon mediums containing a carbohydrate, whereas *B. niger* blackened only mediums containing tyrosine. Attempts to develop nonpigmented dissociants by aging in acid, neutral, and alkaline nutrient broth were not successful. Later, in discussing these results, Gibson[6] told the writers that certain of his freshly isolated cultures of *B. aterrimus* had lost their ability to form pigment after growing in glucose broth. To confirm Gibson's statement, *B. aterrimus* No. 740 was transferred serially in glucose broth at 28° C. Occasionally a culture was obtained that was not black when grown on potato, but if it was cultured again on potato it would revert to the black form.

In an attempt to procure stable variants, a procedure involving the selection of colonies was tried. A blackened potato culture was plated on glucose-nitrate agar, and several of the least pigmented colonies were picked to slopes of the same medium. Those showing the least pigment were transferred again to potato plugs. Of the potato cultures of May 15 (table 11), four were black and one pink. This pink culture was plated on glucose-nitrate agar; colonies were again selected and subcultured on agar slopes and

TABLE 11.—*Loss of black pigmentation of Bacillus aterrimus No. 740 by plating and selection, 1941*

Time of testing	Color of cultures on potato[1]			
	Black	Gray	Pink	Cream
	Number	*Number*	*Number*	*Number*
May 1	*1	----------	----------	----------
15	4	0	*1	0
26	5	0	*2	0
June 9	5	0	*5	0
23	2	4	4	*3
July 5	0	3	6	*3
15	0	3	5	*2
Aug. 6	0	3	2	*6
18	0	0	0	*10
28	0	0	0	12

[1] One of the starred (*) potato cultures was selected each time for plating.

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[6] By oral communication, September 1939.

next on potato. This time five black and two pink cultures resulted. One of the pink cultures was plated, and the selective process was repeated. In this way the black cultures were gradually eliminated. The cream-colored cultures of August 18 and 28 did not revert to the black form during a year's observation.

The stages of dissociation of *B. aterrimus* No. 740 are shown in plate 1, *B*. These four potato cultures represent all the pigments observed—black, gray, pink, and cream.

The ease with which dissociation of *B. aterrimus* can be accomplished varies with the strains selected, as illustrated in table 12. In this experiment the procedure differed slightly from that in table 11. Six strains were transferred serially 50 times on slopes of glucose-nitrate agar and then plated on the same medium. Colonies were selected and transferred to potato plugs. The potato culture showing the least pigmentation was plated again on glucose-nitrate agar and the process repeated. Culture No. 624 lost its black pigment entirely during the serial transfer on glucose-nitrate agar, whereas the black and gray pigments of Nos. 259 and 261 were eliminated only after 2 and 3 platings, respectively. The other 3 cultures showed varying degrees of dissociation. Number 276 reverted to the black form and Nos. 230 and 275 to the gray.

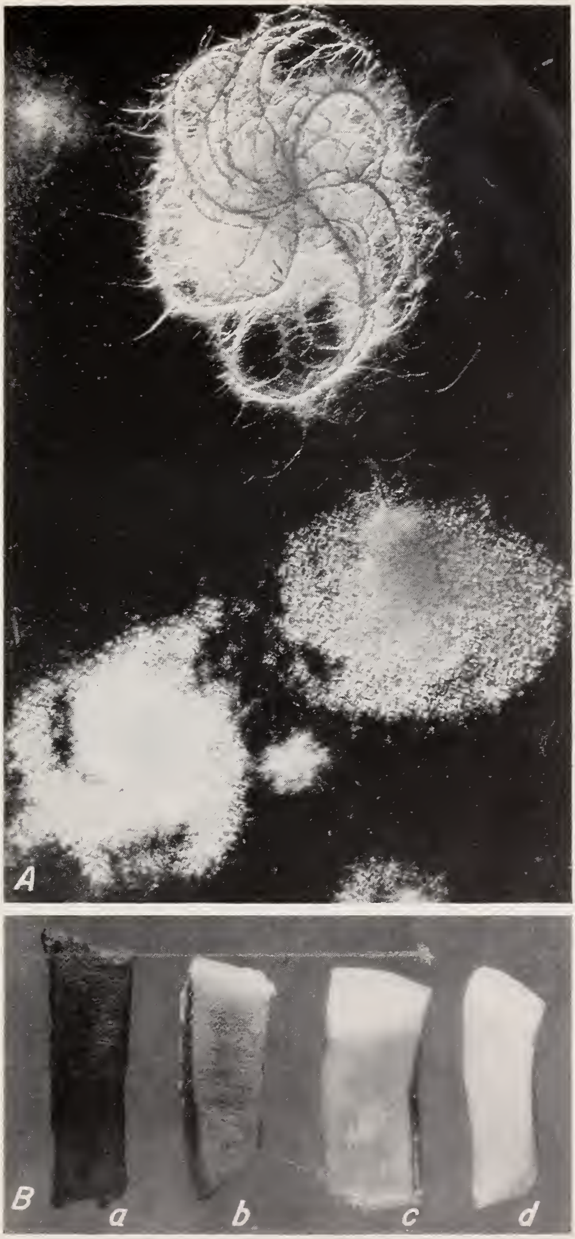
TABLE 12.—*Variation of pigmentation of cultures of Bacillus aterrimus after 50 serial transfers on glucose-nitrate agar, followed by plating, colony selection, and growth on potato, 1942*

Cultures of *B. aterrimus*	Pigment on potato[1] after—											
	First plating, Jan. 17				Second plating, Feb. 2				Third plating, Feb. 12			
	Black	Gray	Pink	Cream	Black	Gray	Pink	Cream	Black	Gray	Pink	Cream
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
No. 230	0	0	0	*8	0	2	0	*4	0	2	0	3
No. 259	1	0	*3	0	0	0	*6	0	0	0	6	0
No. 261	0	1	*3	0	0	3	*3	0	0	0	2	3
No. 275	2	2	0	*2	0	2	0	*4	0	3	0	2
No. 276	0	*4	0	0	*6	0	0	0	2	1	1	0
No. 624	0	0	*8	0	0	0	*6	0	0	0	5	0

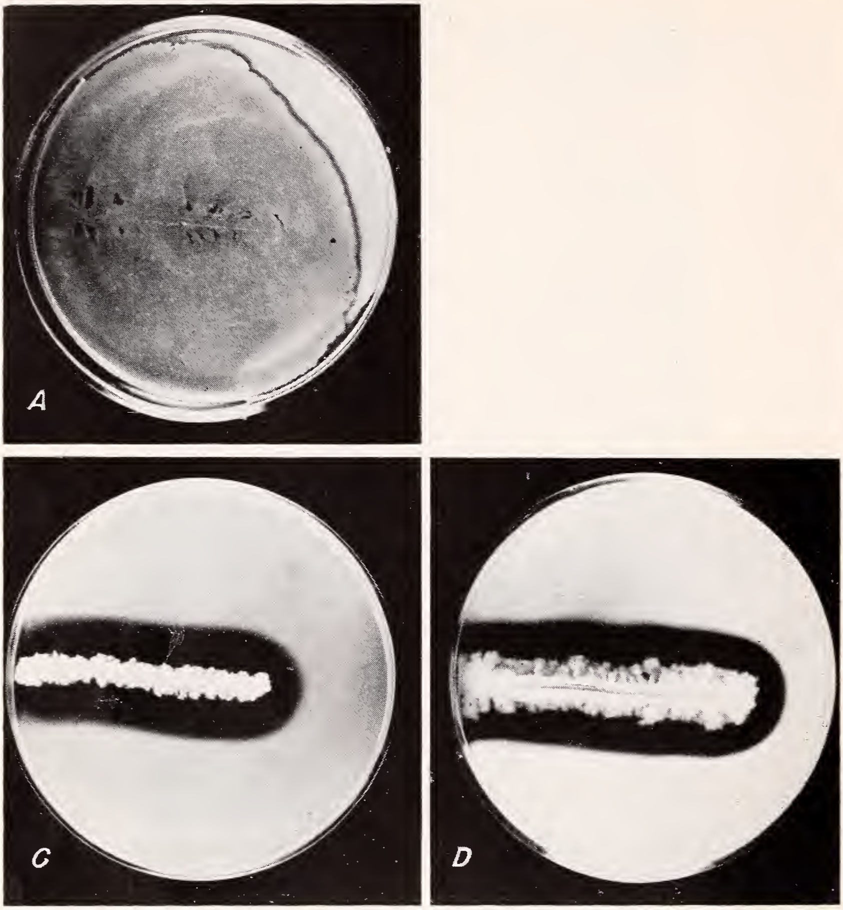
[1] One of the starred (*) potato cultures was selected for replating.

Dissociation of Bacillus niger

Procedures differing from the foregoing ones were followed for dissociating *Bacillus niger*, mainly to show that other techniques may be used. Slants of nutrient agar containing 0.05 percent of tyrosine were inoculated and incubated for 2 weeks at 43° C. At the end of that time, duplicate tubes were inoculated, one placed at 28° and the other held at 43°. After 2 weeks, the process



A, Dissociation of *Bacillus mycoides:* Plate from a 10-day-old broth culture, showing typical colony above and variant colonies below. *B*, Range of pigmentation on potato found in dissociating cultures of *B. aterrimus: a*, Typical black pigment; *b*, gray; *c*, pink; *d*, cream-colored.



Streak plate cultures: *A*, *Bacillus subtilis* on starch-nutrient agar, inoculated as soon as the agar had set; plate flooded with 95 percent alcohol after 1 day at 32° C. to bring out the enzymic zone; *B*, same as *A*, except the poured plate was allowed to dry at room temperature for 2 days before inoculation; *C*, *B. cereus* on gelatin-nutrient agar at 28° C., plate flooded with acid-mercuric chloride solution after 1 day to bring out the enzymic zone; *D*, *B. cereus* on milk agar, showing the zone of hydrolysis of the casein after 2 days at 28° C. (⅔ natural size.)

was repeated, always using the culture from the higher temperature as the inoculum.

Three of the 12 strains tested (Nos. 223, 229, and 650) lost their ability to form the pigment after 12 to 18 transfers. During the same time several other strains definitely showed the inhibitive effect of the higher temperature on pigmentation, even after the first or second transfer. From these, however, stable colorless variants were not obtained.

Another procedure was also tried. Glucose-nutrient broth cultures were transferred serially at 43° C. from 2 to 8 times and plated on tyrosine-nutrient agar. Colonies were picked and incubated at 28°. Three cultures (Nos. 356, 651, and 655) responded to this treatment, whereas five did not lose the pigment during this short test. Owing to interruption of the work, the three colorless dissociants were not fully tested for reversion; it is believed, however, that they were stable.

In the development of the above dissociants, the intermediate cultures, with one exception, merely lost the pigment gradually without going through gradations of pink, as noted with *B. aterrimus.* In the case of No. 356, however, an orange-colored intermediate appeared. It was not stable, giving rise to the usual cream-colored growth typical of the dissociants and of *B. subtilis.* Whether pink, red, or orange intermediates have any bearing upon the nature of the pigment cannot be stated at this time. Muschel (*116*) thought that the pigment of *B. aterrimus* resulted from a polyphenyloxydase blackening of carbohydrate-condensation products. It might differ, therefore, from the melaninelike pigment of *B. niger*, which is probably formed through the tyrosinase system.

About 60 tests were performed in studying the cultures of *B. aterrimus, B. niger,* and *B. subtilis,* and no property, other than pigmentation, that could be used to separate them was found. Without the ability to blacken their substrates, the 52 colorless dissociants developed from 19 strains of *B. aterrimus* and *B. niger* could not be differentiated from *B. subtilis.* It is recommended, therefore, that *B. aterrimus* and *B. niger* be reduced from species rank to that of variety and that they be known as *B. subtilis* var. *aterrimus* and *B. subtilis* var. *niger,* respectively.

FERMENTATION STUDIES

In a preliminary report on the carbohydrate fermentations by members of the genus *Bacillus* (*30*), it was concluded that with only a few exceptions species placed in the same subgroup on the basis of other properties attacked the same carbohydrates. It was also observed that a species might vary in its utilization of a single carbohydrate. Certain strains of *B. megaterium,* for example, readily fermented mannose, whereas others did not. In some cases the negative cultures upon aging on mannose-agar slants gave rise to papillae that produced acidity. Subculturing these papillae yielded a mannose-positive strain. Similar phenomena have often been observed by other investigators (*73, 87, 88*). It was the purpose of the following experiments to study such variations and to learn, if possible, how much taxonomic reliance could be placed on carbohydrate utilization.

It is well known that many of these bacilli, because of their strong proteolytic nature, will not form enough acid from a carbohydrate to overcome the alkalinity produced from organic nitrogen. Consequently, an inorganic nitrogen medium was used in the routine test for determining the availability of a carbohydrate as the only source of carbon. (See section on Mediums and Methods.)

Induced Utilization of Sucrose

Of 109 strains of *Bacillus cereus* 77 were sucrose-positive and 32 were sucrose-negative according to the routine test. All of the negative strains were induced to ferment sucrose by the following procedure: Slopes of the basal agar containing sucrose and an indicator were inoculated rather heavily from nutrient agar slopes and incubated at 28° C. for 1 month. Then as much as possible of the material on each slant was again transferred to a slope of fresh sucrose medium and incubated another month. Some of the cultures showed clearly by a slight growth that they were about to hydrolyze the carbohydrate on the next passage, whereas others gave no indication that they were ready to hydrolyze the sucrose. Acid was formed either by single colonies or uniformly over the slant, indicating in the latter case that many positive cells had been brought over on the last transfer. At the end of the second month 23 of the 32 negative cultures had grown well and formed acid from sucrose (table 13). After another passage they fermented the sugar as rapidly as any of the original sucrose-positive strains. The remaining 9 cultures required from 3 to 7 monthly transfers on the sucrose agar before they attacked that carbohydrate.

As each culture became sucrose-positive it was carefully examined to eliminate any possibility of contamination. Some were then subcultured on nutrient agar and added to the stock collection. After varying periods of storage they were transferred to the sucrose basal medium and observed for acid formation. The results are shown in the last 2 columns of table 13. Although most of the cultures were still sucrose-positive at the end of each storage period, some had already reverted and no longer formed acid from the sugar. Seventeen sucrose-negative strains of *B. cereus* became positive after 2 monthly transfers and remained so after 11 months' storage on nutrient agar; 4 others that became positive at the same time were again negative after the storage period.

The two sucrose-negative strains of *B. brevis* were positive after four and nine transfers, respectively. The first strain was still positive after 10 months, but the latter had become negative again after 4 months. The cultures of *B. laterosporus* were discarded as negative after 6 months, because the initial inoculum had dwindled with each transfer until there was nothing visible on the slopes.

TABLE 13.—*Utilization of carbohydrates by negative strains after monthly serial passage on basal agar containing the specific carbohydrate, and the effect on the induced character of storage on nutrient agar*

Species	Routine test		Negative strains transferred	Monthly transfers	Acid production	Stored on nutrient agar	Acid production after storage
	Positive strains	Negative strains					
SUCROSE FERMENTATION							
	Number	*Number*	*Number*	*Number*		*Months*	
B. cereus	77	32	17	2	+	11	+
			4	2	+	11	−
			2	2	+	----------	----------
			4	3	+	10	+
			1	4	+	9	+
			1	6	+	7	−
			1	7	+	6	+
			1	7	+	6	−
			1	7	+	----------	----------
B. brevis	30	2	1	4	+	10	+
			1	9	+	4	−
B. laterosporus	0	8	8	6	−	----------	----------
MANNOSE FERMENTATION							
B. megaterium	46	25	8	2	+	----------	----------
			5	3	+	6	−
			2	3	+	6	+
			1	3	+	----------	----------
			1	4	+	5	+
			1	4	+	5	−
			4	7	+	----------	----------
			3	7	−	----------	----------
LACTOSE FERMENTATION							
B. cereus	5	93	3	6	+	5	−
			1	6	+	5	+
			1	7	+	3	−
			26	9	−	----------	----------
ARABINOSE FERMENTATION							
B. circulans	31	5	3	4	+	2	+
			1	4	+	2	−
B. cereus	0	123	3	3	−	----------	----------
			3	7	−	----------	----------
B. brevis	0	33	4	7	−	----------	----------
XYLOSE FERMENTATION							
B. alvei	0	9	9	6	−	----------	----------
B. brevis	0	34	1	4	−	----------	----------
			3	6	−	----------	----------
B. cereus	0	123	4	9	−	----------	----------

Table 13.—*Utilization of carbohydrates by negative strains after monthly serial passage on basal agar containing the specific carbohydrate, and the effect on the induced character of storage on nutrient agar*—Continued

GALACTOSE FERMENTATION

Species	Routine test		Negative strains transferred	Monthly transfers	Acid production	Stored on nutrient agar	Acid production after storage
	Positive strains	Negative strains					
	Number	*Number*	*Number*	*Number*		*Months*	
B. brevis	25	7	3	2	+	----------	----------
			2	3	+	----------	----------
			1	4	+	----------	----------
			1	9	−	----------	----------
RHAMNOSE FERMENTATION							
B. alvei	0	9	9	7	−	----------	----------
B. megaterium	8	36	1	4	+	----------	----------
			1	5	+	----------	----------
			2	11	−	----------	----------
STARCH FERMENTATION							
B. pumilus	0	69	12	7	−	----------	----------
B. brevis	0	33	3	5	−	----------	----------
B. laterosporus	0	7	3	5	−	----------	----------
GLYCEROL FERMENTATION							
B. brevis	19	10	2	2	+	10	+
			1	2	+	10	−
			1	3	+	9	+
			3	4	+	8	+
			1	4	+	8	−
			1	10	+	----------	----------
			1	11	−	----------	----------

INDUCED UTILIZATION OF OTHER CARBOHYDRATES

To induce fermentation of mannose, 25 mannose-negative strains of *Bacillus megaterium* were subjected to the same procedure as that used above. All except 3 became positive after 2 to 7 transfers (table 13). After storage for 5 or 6 months in the absence of the carbohydrate, 6 strains of the 9 tested were again negative. Similar results were shown by *B. brevis* on glycerol.

The fermentation of lactose by *B. cereus* was not common; only 5 of 98 strains were positive by the routine test. Of the negative strains, 31 were transferred serially, 5 becoming positive in 6 or 7 months. Only 1 of these, however, retained this property after

5 months' storage on nutrient agar. Similar results were obtained with *B. megaterium* on rhamnose. Considering all the foregoing data, there seems to be no relation between the length of time required for the development of the ability to use a carbohydrate and the stability of that character.

The routine tests using *B. cereus* (with the exception of Nos. 248 and 1332) and *B. brevis* were not positive on arabinose and xylose, nor were any of the 18 strains selected induced to use those carbohydrates. Similar results were obtained with *B. laterosporus* on sucrose, *B. alvei* on xylose and rhamnose, and *B. pumilus, B. brevis,* and *B. laterosporus* on starch.

To summarize, a total of 82 cultures were induced to use a carbohydrate that was not fermented previously in the routine test. Of the 57 cultures kept under observation, 20 (35 percent) lost this acquired ability after storage on nutrient agar.

In this work on fermentation, it was not possible to study further these "trained" enzymes and the problems connected with them. They did not seem to fulfill Dubos' specifications of an adaptive enzyme (*48*), nor were they apparently so stable as the lactase of *Escherichia coli-mutabile* described by Lewis (*103*). Their reversion did not appear to depend on the time required for adaptation, as observed by Hinshelwood and Jackson (*73*) in the case of *Bacterium lactis aerogenes.* They seemed, however, to be much like certain enzymes of several types of the dysentery bacilli reported by Courmont and Rochaix (*39, 40*). After repeated transfer on a medium containing the particular substance, their strains would ferment a carbohydrate that was normally not attacked. This new fermentation capacity frequently proved to be only temporary.

Value of Fermentation Tests

Fermentation tests on members of the genus *Bacillus* had a taxonomic value, provided they were interpreted liberally. There was no doubt of their importance where all strains of a species were either positive or negative, but where various percentages were positive a problem was presented. It was found that if most of the strains were positive, most of the others might be induced to utilize the carbohydrate, although this acquired character might not be stable. On the other hand, if only a small percentage of the strains of a species were positive, there was little chance of inducing the utilization of a carbohydrate by the negative cultures. It was concluded that the few positive strains deserved recognition as biotypes.

TEMPERATURE RELATIONSHIPS OF SPECIES

Sporeforming bacteria capable of growth at high temperatures have been characterized by various workers in a number of ways. Prickett (*128*) accepted the following definition of Cameron and Esty (*21*): Cultures growing at 55° C. but not at 37° are classified as obligate thermophiles and those growing at both 55° and 37° as facultative thermophiles.

In an attempt to apply this definition to the strains of this collection, growth at temperatures of 28° C. to 70°, inclusive, was determined for a number of cultures of several species (*65*). The results were arranged (table 14) to show the decrease in the upper limits for growth of the various species. Approximately 50 percent of the cultures of *Bacillus stearothermophilus* grew at 70° and all grew at 65°. On the other hand, none of the strains of *B. coagulans* grew at 65°. All strains of *B. subtilis, B. brevis,* and *B. circulans* failed at 60°; *B. pumilus* and *B. macerans,* at 55°; and *B. cereus* and *B. sphaericus,* at 50°. With the exception of *B. stearothermophilus* and *B. coagulans,* all cultures of the species listed above grew at 28°.

TABLE 14.—*Growth temperatures of several species of aerobic sporeforming bacteria*

Species	Number of cultures	Number of cultures growing at[1]—								
		28° C.	33° C.	37° C.	45° C.	50° C.	55° C.	60° C.	65° C.	70° C.
B. stearothermophilus	87	0	10	73	81	87	87	87	87	45
B. coagulans	73	53	73	73	73	72	66	23	0	------
B. subtilis	154	154	154	154	150	105	17	0	------	------
B. brevis	57	57	57	57	38	16	7	0	------	------
B. circulans	55	55	55	51	18	6	1	0	------	------
B. pumilus	65	65	65	65	64	43	0	------	------	------
B. macerans	13	13	13	13	13	9	0	------	------	------
B. cereus	50	50	50	50	23	0	------	------	------	------
B. sphaericus	42	42	42	42	15	0	------	------	------	------

[1] Water-bath temperatures were used for all temperatures except 28° C.

It was apparent that the temperatures of 55° and 37° C., selected by Cameron and Esty (*21*) for the classification of thermophilic sporeformers, could not be used to differentiate species. Of the higher temperatures it seemed that only 65° might possibly be used to separate *B. stearothermophilus* from *B. coagulans.* Approximately one-third of the strains of *B. coagulans,* however, grew at 60°, leaving only a 5° margin for the separation of the two species.

Beside providing a comparison of the maximum temperatures permitting growth of the various species, table 14 shows the great differences among strains of a species with respect to their highest temperatures for growth. Although 4 of 154 strains of *B. subtilis,* for example, failed to grow at 45° C., 17 grew at 55°. From a taxonomic standpoint, therefore, little reliance could be put on a strain's maximum temperature of growth for its identification.

In this connection the question might be raised as to whether those strains of a species having a relatively low maximum temperature for growth might be acclimatized to grow at a higher temperature. A few unsuccessful attempts made by the writers along this line and the work of Casman and Rettger (*23*) indicate that the maximum temperature for growth of a culture is not likely to change. In experiments of a year's duration Casman and Rettger attempted to induce members of the "subtilis group" to grow at higher temperatures. When cultivated on agar slants their strain F of *B. subtilis,* for example, grew at a maximum temperature of 50° to 60° C. and strain T at a maximum of 49° to 50°, or 10° lower than strain F. Neither strain F nor strain T could be induced to grow at higher temperatures.

The overlapping of temperature ranges of growth of the various species present in this collection (table 14) made it impossible to use temperatures for growth as a criterion for separation of these species. Exception was made in the case of *B. stearothermophilus,* but the margin of difference was so small in this instance that it could be regarded with suspicion. How, then, should the term "thermophilic" be applied? The writers suggest it be used in a general way to denote cultures capable of growth at 55° C., in accordance with Cameron and Esty's definition, but never to describe a species. It is recognized that a culture's ability to grow at 55° is of more interest, for example, to investigators in the canning industry than its species identification and that the term "thermophilic" has recognized descriptive value. It is believed, therefore, that the recommended restriction of the term to cultures growing at 55° offers the best solution available and that the avoidance of its use in the taxonomy of species will tend to lessen further confusion over the identity of species of the genus *Bacillus.*

REJUVENATION OF ABERRANT CULTURES

During the study of the various species, certain strains were encountered whose species patterns differed from those of recognized species in being negative in one or more characters. In 1946 (*147*) those cultures were considered as intermediates, but subsequent work has led the writers to believe that negative characters without compensating positive characters should not be diagnostic and that such strains should more properly be called weak or aberrant rather than intermediate. In the section Studies on Dissociation it was shown that the mycoid type of growth might be lost during cultivation in broth and that pigmentation could be eliminated by colony selection and other means. It was logical, therefore, to postulate that certain strains might lose important characteristics either spontaneously or as a result of unfavorable conditions, thus appearing weak or aberrant, and that some of them could be rejuvenated to the normal form.

Aberrant Strains of Bacillus megaterium

It has been shown (table 13) that 22 of 25 mannose-negative strains of *Bacillus megaterium* became positive after 2 to 7 monthly serial transfers on the mannose basal medium. Likewise, 2 of 4 cultures negative on rhamnose became positive, but the other 2 persisted in remaining negative.

In response to the technique used in inducing utilization of carbohydrates, cultures Nos. 838B and 839B, which were received as *B. cohaerens* (see the assigned cultures of *B. megaterium*) grew well on glucose-nitrate agar only after seven serial transfers. Culture No. 994 (in the synonymy) also grew poorly in the routine test, but upon aging a secondary colony of this culture appeared on the slope of the glucose-nitrate agar slant, which after being transferred to a fresh slant of the same medium produced the typical slimy growth. Other instances were observed in which good growth followed serial transfer on a certain medium, although only a poor growth was evident in the routine test. This was especially true of four strains (Nos. 1071, 1072, 1080, and 1084 in the list of unnamed cultures) that possessed the general pattern of *B. megaterium*. They grew poorly, if at all, on soybean agar, but after five serial transfers all grew abundantly. These four cultures were also negative on arabinose and xylose and remained so even after nine serial transfers on these carbohydrates. They were next inoculated into N-Z case broth (trypsin digest of casein) and it was noted that No. 1080 grew much better than the others. When arabinose and xylose basal agar slants were inoculated from these broth cultures, No. 1080 was found to hyrolyze both pentoses, whereas the other three still remained negative. In spite of this the three pentose-negative cultures have been included in the species, because it was felt that the right method was not used to develop their normal growth and physiology.

It was impossible to attempt to rejuvenate all the *B. megaterium-B. cereus* intermediates (*147*) because of lack of assistance. A few tests were made, however, such as serial transfer in V-P broth, on soybean agar, and on glucose-nitrate agar, but none of the cultures other than those mentioned changed from their original pattern.

Aberrant Strains of Bacillus cereus

It was shown in the section on Fermentation Studies that 32 of 109 strains of *Bacillus cereus* were negative on sucrose, according to the routine test. All became positive after 2 to 7 monthly serial transfers on the sucrose basal medium. On the other hand, 93 of 98 strains failed to ferment lactose and only a few of the lactose-negative strains became positive after serial transfer. It was concluded that, if the majority of the strains of a species fermented a carbohydrate, those that were negative might be induced to become positive, but that, if most of the cultures were negative, there was little chance of changing the negative ones.

Because nearly all the cultures of *B. cereus* formed acetoin, it was decided to transfer three V-P negative cultures (Nos. 411, 600, and 1261 in the list of unnamed cultures) serially in V-P broth. This attempt was not successful, however; all failed to produce any acetoin. Later, No. 1261 was subcultured in V-P broth from an old culture on potato and a 2+ test for acetoin was obtained. This result was checked by inoculating a potato plug from the stock culture of strain No. 1261. Again, broth inoculated from the potato culture was V-P positive, whereas one made directly from the stock was negative. This does not mean that other negative cultures would react in the same way. It does indicate, however, that cultures having the general species pattern of *B. cereus* but failing to produce acetoin may be considered as weakened or aberrant strains of that species rather than as intermediates between *B. cereus* (V-P positive) and *B. megaterium* (V-P negative).

Aberrant Strains of Bacillus subtilis

A few strains of *Bacillus subtilis* failed to reduce nitrates to nitrites or to produce acetoin in the routine test. After 9 to 14 weekly serial transfers into nitrate-nutrient broth, strain No. 263 (*B. aterrimus* in the assigned cultures of *B. subtilis*) and No. 714 (an unnamed culture) gave a strongly positive reaction for nitrites. The experiment was repeated twice with No. 263, with the same results.

Serial transfer into V-P broth was effective in rejuvenating strains No. 1243 and 1244 (unnamed cultures). Number 1243 showed a trace of acetoin in the seventh transfer and a 2+ in in the fourteenth. On the other hand, No. 1244 failed to show any acetoin until the fourteenth transfer. A 3+ reaction was observed on the next transfer. Both of these strains were serially transferred on soybean agar at the same time the tests were made and V-P broth inoculated from each soybean tube. No acetoin was produced in any of the 15 subcultures.

Allocation of Aberrant Strains

In the attempt to identify a large number of strains of the genus *Bacillus,* a small percentage of the cultures did not strictly conform to a recognized species pattern, usually because of a lack of positive characteristics. When only one or two reactions were involved, it was sometimes possible to rejuvenate the culture by serial transfer or by cultivation in some especially favorable medium prior to the final test. Certain strains, however, failed to respond to any treatment that was given and remained aberrant. Such strains, if they bore specific names, were placed in the unclassified list.

In some cases only one of two or three apparently identical cultures responded to the particular treatment. What the explanation is can only be conjectured. It might be that the unresponsive cultures have become stabilized and really represent mutations involving the loss of characters.

Strains having a positive character in addition to those of a certain species pattern were rarely encountered. They may be regarded as true intermediates or as biotypes, whichever term is preferred. For example, *B. cereus* No. 248 (list of unnamed cultures) proved typical for the species, except that it fermented xylose. This and the very few others like it have been allocated to the logical species, with the notation that they are considered as biotypes.

MEDIUMS AND METHODS

The microscopic appearance of these organisms was found to be of prime importance in dividing the genus into three major groups. Smears of young cultures grown on nutrient agar were stained by aqueous fuchsin (0.5 to 1.0 ml. of a saturated solution of basic fuchsin in 100 ml. of distilled water) to determine the size of the rods and the formation of shadow-forms (ghosts), capsules, and chains. Gram's reaction and motility were also observed. Cells grown on glucose-nutrient agar for 2 to 3 days were lightly stained, and the presence of vacuoles in the protoplasm was recorded. (See section on Storage of Fat.) Reserve fat was demonstrated by Hartman's method (*71*) by mixing a loopful of the culture on a microscope slide with a drop of ethylene glycol saturated with Sudan black B. Fat droplets were stained dark blue or black.

The size and shape of the mature spores and the shape of the sporangium proved valuable points for separating the species into three groups. It should be born in mind, however, that complete reliance on any one character is not to be expected. One observer might think that a sporangium was definitely swollen, whereas another would recognize the fact that part of the vegetative rod had shrunken, giving a false impression of bulging. In case of doubt, it might be best to compare the diameter of the mature spores with that of the young vegetative rods. The time of sporulation, the relative stainability of the spore wall with aqueous fuchsin, and the abundance of spores on various mediums were also noted.

Routinely, the general appearance and size of colonies on nutrient agar and the quantity and character of growth on various mediums were described. The latter was often of considerable help to the trained observer in the recognition of species. It must be pointed out, however, that the gross morphology of these cultures has long been known to be exceptionally variable.

Although many of the mediums used in this work are common in most laboratories, for the sake of completeness all are listed below. Unless otherwise noted, sterilization was by autoclaving for 20 minutes at 121° C.

Nutrient agar.—Peptone, 5 gm.; beef extract, 3 gm.; agar, 15 gm.; distilled water, 1,000 ml.; pH 7.0.

Nutrient broth.—The same as above without the agar.

NaCl broth.—Nutrient broth with varying percentages of NaCl.

Glucose-nutrient agar.—Nutrient agar with 1 percent glucose.

Beef-extract agar.—Beef extract, 5 gm.; agar, 15 gm.; distilled water, 1,000 ml.

Tyrosine agar.—Nutrient agar with 0.1 percent tyrosine.

Citrate agar.—NaCl, 1.0 gm.; $MgSO_4$, 0.2 gm.; $(NH_4)_2HPO_4$, 1.0 gm.; KH_2PO_4, 0.5 gm.; Na citrate, 2.0 gm.; agar, 15 gm.; distilled water, 1,000 ml.; and 20 ml. of a 0.04 percent phenol red solution (pH adjusted to 7.0 before the addition of the indicator). This formula is a modification of Koser's citrate ammonium phosphate solution (*86*).

Potato plugs.—Cores, 10 to 15 mm. in diameter, were cut from potatoes and autoclaved with a few ml. of distilled water in small screw-capped bottles or potato tubes. Due allowance was made for the variability in the composition of potatoes in interpreting the observations.

Soybean agar.—One hundred grams of yellow soybeans were soaked overnight in 1,200 ml. of distilled water, steamed for 90 minutes, and then filtered through cotton. The broth was made up to 1,000 ml.; 0.5 gm. of K_2HPO_4 and 15 gm. of agar were added and dissolved, and the pH adjusted to 6.8 (*168*).

Proteose-peptone acid agar.—Proteose-peptone, 5 gm.; yeast extract, 5 gm.; glucose, 5 gm.; KH_2PO_4, 4 gm.; distilled water, 500 ml.; pH 5.0. An equal volume of 2.5 percent agar, pH 5.0, was prepared and autoclaved separately. After sterilization the two portions were cooled to about 50° C., mixed, tubed asceptically, and slanted (*158*).

Stock culture agar.—Difco stock culture medium (*2*) solidified by the addition of 7.5 gm. of agar per 1,000 ml.

Glucose-nitrate agar.—K_2HPO_4, 1 gm.; $NaNO_3$ 1 gm.; agar, 15 gm.; distilled water, 1,000 ml.; glucose, 10 gm.

Glucose-asparagine agar.—Asparagine, 0.5 gm.; K_2HPO_4, 0.5 gm.; agar, 15 gm.; glucose, 10 gm.; pH 1.0 (modification of Conn's glycerol-asparaginate agar (*35*)).

Tomato-yeast milk.—Difco skim milk, 100 gm.; yeast extract, 5 gm.; distilled water, 1,000 ml.; tomato juice, 100 ml. The pH of the tomato juice was adjusted to 7.0 before its addition to the other ingredients.[7] The medium was tubed and sterilized in a water bath in the autoclave for 12 minutes at 121° C. (Certain batches of this medium were not so satisfactory as others, for some unknown reason. Equally erratic were the results when the juice of canned tomatoes was used and the medium sterilized in the usual manner.)

Hydrolysis of starch.—Cultures were streaked once across plates of nutrient agar containing 1 percent of potato starch. (Filtration of the agar, when necessary, was done before addition of starch.) After incubating from 1 to 8 days, depending on the growth rate of the organism, the plates were flooded with 95-percent alcohol. If the starch was hydrolyzed a translucent zone appeared around or underneath the growth (*83, pl. 1, fig. 2*), but if it remained unchanged the medium became white and opaque. The spreading of some cultures over the plate was inhibited by storing the plates 2 or 3 days before inoculation (pl. 2, *A* and *B*.)

Production of acetylmethylcarbinol.—The medium recommended by the Manual of Methods (*150*) and Standard Methods (*1*) for the Voges-Proskauer reaction was modified after experiments had shown that phosphates inhibited the formation of acetoin by some of these organisms. (See section on Factors Affecting the Production of Acetylmethylcarbinol.) The medium used contained 7 gm. of proteose-peptone, 5 gm. of glucose, 5 gm. of NaCl, and 1,000 ml. of distilled water. Five-milliliter portions in 18-mm. tubes were inoculated and incubated at a suitable temperature for 2, 4, and 6 days, or similar intervals, and occasionally for 10 and 20 days. The test was made by mixing the culture with an equal volume of a 40-percent solution of NaOH and adding 0.5 to 1.0 mg. of creatine from a knife point. The appearance of a red color in the culture after it had stood 30 to 60 minutes demonstrated the presence of acetoin (*123*).

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[7] Personal communication, R. P. Tittsler, U. S. Department of Agriculture, 1944.

pH of glucose broth.—Before the 6- or 7-day-old cultures were tested for acetylmethylcarbinol, about 1 ml. was withdrawn and the pH determined by the colorimetric method.

Fermentation tests.—For the fermentation studies a basal medium was used, a modification of one proposed by Ayers, Rupp, and Johnson (*3, p. 13*). The formula was as follows: $(NH_4)_2HPO_4$, 1.0 gm.; KCl, 0.2 gm.; $MgSO_4$, 0.2 gm.; yeast extract, 0.2 gm.; agar, 15 gm.; and distilled water, 1,000 ml. Twenty milliliters of a 0.04-percent solution of bromcresol purple was added as the indicator and the medium was tubed and autoclaved. A 10- or 15-percent aqueous solution of the test carbohydrate, sterilized separately by autoclaving or filtration through an L2 Pasteur-Chamberland filter, was pipetted into each tube in such quantity as to result in a 0.5-percent concentration of the carbohydrate. The tubes were incubated overnight, examined for sterility, inoculated, and observed for growth, acid, and gas at intervals up to 10 or 20 days.

If a strain failed to hydrolyze glucose in this basal carbohydrate medium, it was inoculated on slopes of glucose-nutrient agar with an indicator, usually bromcresol purple. If acid was then formed, it was assumed that the strain could not utilize the ammoniacal nitrogen in the basal medium and required organic nitrogen. Unless otherwise indicated, the basal carbohydrate medium was used in this work.

Crystalline dextrins.—The formation of crystalline dextrins from starch was detected by an adaption of the iodine test of Tilden and Hudson (*163*). The cultures were incubated at 37° C. in rolled-wheat and wheat-grain mashes, because they proved more satisfactory than oatmeal mash. About 0.5 gm. of rolled wheat, 0.2 gm. of $CaCO_3$, and 15 ml. of distilled water were placed in large test tubes and sterilized. The other medium consisted of several grains of wheat, about 0.5 gm. of $Ca_3(PO_4)_2$, and 15 ml. of distilled water. After 3, 6, and 10 days of incubation a drop of the clear supernatant liquid of the culture and a drop of Lugol's iodine were mixed on a clean microscope slide and allowed to dry. If crystalline dextrins were present in small amounts, brown to blue hexagonal crystals were demonstrable (magnification, × 100) at the edge of the film. If these dextrins were present in larger amounts, long crystalline needles in fan-like arrangements also appeared at the edge and extended into the film (pl. 3, *E*).

In some cases what seemed to be long filaments were observed around the edge of the film. These were not crystalline dextrins, but cleavage lines caused by the presence of noncrystalline products. This method was found to be more sensitive and simpler than the technique used by Tilden and Hudson (*163*).

Hydrolysis of gelatin.—Plates of nutrient agar with 0.4 percent gelatin were streaked once across and incubated at a suitable temperature. After 3 to 5 days the plates were flooded with 8 to 10 ml. of the following solution (*56*): $HgCl_2$, 15 gm.; concentrated HCl, 20 ml.; distilled water, 100 ml. The extent of hydrolysis of the gelatin was indicated by a clear zone underneath and around the growth, in contrast to the white opaque precipitate of the unchanged gelatin (pl. 2, *C*). As in the case of the starch plates, the spreading of some cultures could be checked by storing the plates for several days before inoculation (pl. 2, *A* and *B*).

Hydrolysis of casein.—Milk agar was prepared by mixing hot sterile 2.5-percent water agar with half as much cool sterile skim milk. Plates were quickly poured and, after solidification, were streaked once across. At various intervals they were observed for growth of the organism and zone of clearing of the casein (pl. 2, *D*). This is a modification of Hastings' method (*72*).

Production of indole.—The cultures were inoculated into 1 percent Difco tryptone and 1 percent BBL trypticase broths (5-ml. amounts in 18-mm. tubes). After 6, 10, and 14 days' incubation at a suitable temperature, usually 32° C., the cultures were shaken with 2 ml. of the following solution (*150*): Paradimethylaminobenzaldehyde, 5 gm.; amyl alcohol, 75 ml.; concentrated HCl, 25 ml. A pink to red color in the alcohol layer indicated the presence of indole.

Urease.—The production of urease was demonstrated by growing the cultures on slopes of nutrient agar and testing for urease after 3 and 7 days. The growth was washed off with 2 ml. of distilled water into a clean test tube. A drop of phenol red indicator was added and the reaction brought to pH 7.0 by a few drops of very dilute HCl or NaOH. The suspension was divided equally between two tubes and approximately 0.1 gm. of crystalline urea was added to one, the other serving as the control. If urease was present the suspension with the urea became very alkaline in a few minutes.

Reduction of nitrate to nitrite.—The method described in the Manual of Methods (*150*) was adopted for this determination. The cultures were grown in nutrient broth containing 0.1 percent KNO_3 and incubated at suitable temperatures. After 1, 3, 6, or more days several drops of each of the following solutions were added to a small portion of the culture: (1) Sulfanilic acid, 8 gm.; 5N acetic acid (1 part glacial acetic acid to 2.5 parts of water), 1,000 ml.; (2) dimethylalphanaphthylamine, 6 ml.; 5N acetic acid, 1,000 ml. The appearance of a deep red color or a heavy yellowish precipitate indicated the presence of nitrite. If the reaction was negative the rest of the culture was tested for nitrates by adding a few drops of diphenylamine and pouring concentrated H_2SO_4 down the side of the tube to form a layer underneath. A blue color at the junction of the two layers was indicative of nitrates (*180*).

Anaerobic production of gas from nitrate.—A medium suggested by Gibson (*63*) was modified for demonstrating anaerobic formation of gas from nitrate. It contained 1.0 percent tryptose, 0.5 percent K_2HPO_4, 0.3 percent beef extract, 0.2 percent yeast extract, and 1.0 percent $NaNO_3$. Approximately 8 ml. of the broth was put into 16-mm. tubes and autoclaved. The pH was about 7.6. Before use the medium was steamed to drive off the free oxygen, quickly cooled, inoculated, capped with 10 to 15 mm. of sterile melted vaspar (equal parts of vaseline and paraffin), and incubated at 32° C. Observation for gas were made at 7 and 14 days.

For species requiring incubation temperatures of 45° or 50° C. the technique had to be modified as follows: Melted vaspar was poured on top of the inoculated medium to a depth of 3 or 4 mm. and allowed to harden. Melted 1.5 percent water agar was then added to a depth of 15 or 20 mm., the tubes cooled, and the culture finally incubated at the high temperatures. Results were usually obtained in 2 or 3 days before the agar developed cracks caused by drying. A layer of vaspar may also be placed on top of the agar.

Anaerobic growth in glucose broth.—The above medium with 1 percent glucose substituted for the $NaNO_3$ was prepared by adding a sterile 50-percent solution of glucose aseptically to each tube. Anaerobiosis and incubation temperatures were the same as those described above. Growth was recorded at 7 and 14 days, and the colorimetric determination of the pH made at the fourteenth day. The pH of the controls was about 7.6. For certain species this was too alkaline and a near neutral or slightly acid medium was necessary.

Reduction of methylene blue.—Glucose, 5 gm.; proteose-peptone, 10 gm.; agar, 2.5 gm.; methylene blue, 0.004 gm.; and distilled water, 1,000 ml. Tubes 15 mm. in diameter containing 8 ml. of the semisolid medium were inoculated and examined for the reduction of methylene blue at 1, 3, 5, 7, 14, and 21 days.

Temperatures for growth.—As most of the strains studied grew well at 28° C., this temperature, unless otherwise noted, was used for the incubation of cultures prepared for all routine tests and observations.

To determine growth at temperatures higher than 37° C., nutrient agar slants were placed immediately after inoculation in a water bath of the desired temperature. After reaching equilibrium they were transferred to a similar water bath kept inside a constant-temperature incubator.

Attention should be called to the fact that, in high-temperature incubators, evaporation, radiation from the heater, stratification of the air, and other conditions alter the actual temperature of the medium on which the micro-organism is growing. The differential between the temperature of the medium and that shown by the air thermometer may be considerable, depending on the characteristic of the incubator. The air thermometer usually being higher than the culture, it is recommended that for accurately measuring temperatures of growth above 37° C. the cultures be incubated in water baths inside an incubator.

Isolation of thermophilic cultures.—The following technique was usually employed for the isolation of thermophilic aerobic cultures (*65*). Small quantities of milk, cheese, cream, soil, or silage were added to tubes of sterile nutrient broth, then heated to 85° C. and held for 5 to 10 minutes. After the preliminary heating the tubes were quickly cooled to 57° and incubated overnight in a water bath at the latter temperature. The broth cultures were then plated on nutrient agar and incubated at 45° or 57° for 24 to 48 hours. Isolated colonies representative of the types present on the plates were selected and transferred to nutrient agar slants.

This method was frequently modified in one or more of the following ways: The material was suspended in glucose or meat-infusion broth; 100° C. for 5 minutes was used instead of 85° to destroy all vegetative forms; the broth suspensions were incubated at 55°, 60°, 62°, or 65° for 24 or 48 hours; and the final plating was made on glucose or starch agar instead of on nutrient agar.

KEY TO SPECIES OF GENUS

The key presented below is based on the descriptions of cultures of the species studied. An effort was made to arrange the species to show their natural relationships, rather than to develop only an artificial key. If all species had definite, unalterable characters a key could be prepared that would always be of value in placing a particular culture. Unfortunately, they do not. It is the writers' experience, and it seems to be more generally recognized as time goes on, that practically any bacterial character may vary. A key based on single characters, therefore, would be of limited value. This key, therefore, uses two or more characters as far as possible at each separation. Should the worker err in his judgment or the observed character be variable, the other character would prevent placing the culture in the wrong group. It is quite possible, for instance, for an investigator to misinterpret his readings in measuring the diameter of the vegetative rods, a difference of 0.2μ allocating his culture to either the *Bacillus cereus* or the *B. subtilis* subgroups. The character of uniformly stained protoplasm would become the deciding element in this case.

The key is intended to provide a guide for the classification of normal, typical strains. It cannot be expected to be of use in the

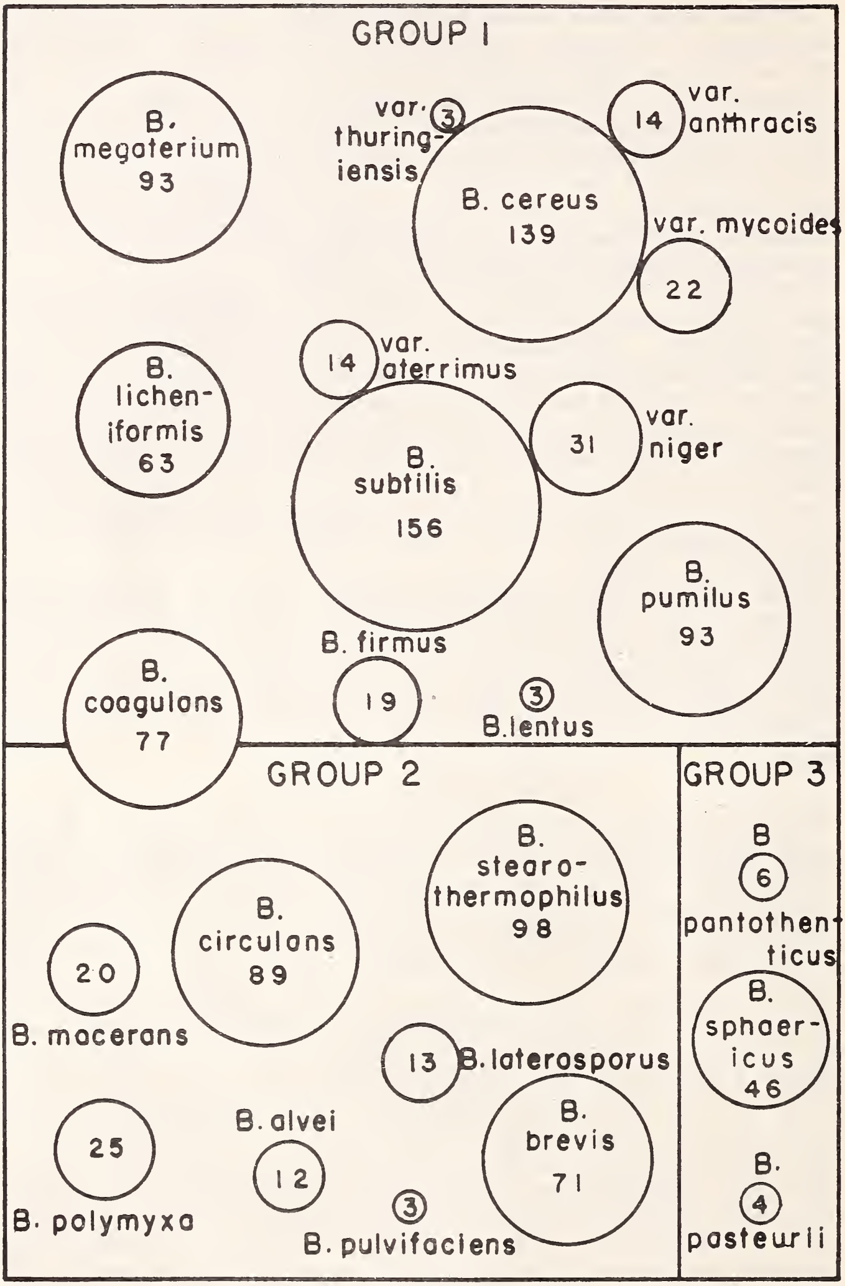

Figure 2.—The numbers and, to some extent, the relationship of the species of *Bacillus* studied. The area of each circle was computed by multiplying the number of strains indicated by 6 mm.^{2}.

allocation of intermediate, aberrant, or otherwise atypical cultures. For definite identification all strains must be studied in detail and the pattern of their characters compared with the patterns of known species.

Should major differences be found between the pattern of a new strain and that of known species, the designation of a new species would be justified. Just what are major differences is a subject on which not many will agree. No specific rules can be laid down to apply to all genera or even to all species in a genus. The writers adopted the criterion that a character must be relatively stable for it to be used in distinguishing a species. Whether it is stable can best be shown by observations on a large number of strains from various sources and by studies on variation. Should strains otherwise alike have a character varying, for instance, from strongly positive to negative and correlating with no other reaction, the writers have considered it merely as a variable. The production of nitrites from nitrates by *B. cereus*, for example, may be very strong to negative; it shows no relationship to any other variable character, such as carbohydrate fermentation, motility, or stage of growth, and is consequently unreliable for purposes of identification. On the other hand, the reduction of nitrates to nitrates by *B. subtilis*, although sometimes weak, was always positive and has been used as one of the properties separating this species from *B. pumilus*. Gibson (*63*), however, noted that 10 out of 122 cultures of *B. subtilis* failed to produce nitrates.

The relative abundance of any one species, as represented in the writers' collection (fig. 2), may or may not have a connection with its occurrence in nature. Group 1 contains about 65 percent of the cultures studied and approximately 62 percent of the unnamed cultures sent to the writers by other investigators for identification. It is evident that certain species in groups 2 and 3 are not adequately represented in this study. Whether they are more or less important in nature than is indicated by the chart cannot be stated. Certainly more cultures should be isolated and studied in order to gain more information on the variability of each species. Studies on dissociation are also needed.

Three recognized mesophilic species of the genus—*B. larvae* White, *B. popilliae* Dutky, and *B. lentimorbus* Dutky (*14*)—have not been included in this investigation. Other species not listed here no doubt will be brought to light by future work. It is the hope of the writers that no new species will be described without a study of an adequate number of cultures and a comparison with existing closely related forms. The key to the species studied follows:

Group 1. Sporangia Not Definitely Swollen

a. Spores oval to cylindrical, central to terminal; spore wall thin; sporangia only slightly swollen if at all;[8] Gram-positive.

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[8] Nearly 50 percent of the strains of *B. coagulans* had definitely swollen sporangia. In most other aspects, it resembled the members of group 1 more than those of group 2 and consequently has been placed in the former.

b. Protoplasm of young cells grown on glucose-nutrient agar vacuolated if lightly stained; diameter of vegetative rods 0.9μ or more.

c. Acid from mannitol in the basal medium; acetylmethylcarbinol not produced .... *B. megaterium.*

cc. No acid from mannitol in the basal medium; acetylmethylcarbinol produced .... *B. cereus.*

1. Rhizoid growth .... *B. cereus* var. *mycoides.*
2. Causative agent of anthrax .... *B. cereus* var. *anthracis.*
3. Pathogenic to insects .... *B. cereus* var. *thuringiensis.*

bb. Protoplasm of young cells grown on glucose-nutrient agar not vacuolated; diameter of vegetative rods less than 0.9μ.

c. Growth on glucose-nutrient agar as good or better than on nutrient agar; good growth on soybean agar.

d. Growth in 5 percent NaCl broth; strong hydrolysis of gelatin.

e. Starch hydrolyzed; nitrates reduced to nitrites.

f. Good growth under anaerobic conditions, pH of glucose broth cultures 5.2 or below; gas from nitrates under alkaline anaerobiosis .... *B. licheniformis.*

ff. Scant if any growth under anaerobic conditions, pH of glucose broth cultures higher than 5.2; no gas from nitrates under alkaline anaerobiosis .... *B. subtilis.*

1. Black pigment on carbohydrate mediums only .... *B. subtilis* var. *aterrimus.*
2. Black pigment on tyrosine mediums only .... *B. subtilis* var. *niger.*

ee. Starch not hydrolyzed; nitrates not reduced to nitrites .... *B. pumilus.*

dd. No growth in 5 percent NaCl broth; weak, if any, hydrolysis of gelatin .... *B. coagulans.*

cc. Growth on glucose-nutrient agar definitely not so good as on nutrient agar; scant, if any, growth on soybean agar.

d. Casein hydrolized; urease not formed .... *B. firmus.*

dd. Casein not hydrolyzed; urease formed .... *B. lentus.*

Group 2. Sporangia Definitely Swollen by Oval Spores

aa. Spores oval, rarely cylindrical, central to terminal; spore wall thick, remnants of sporangium sometimes adhering; sporangia definitely swollen;[8] Gram-variable.

b. Gas formed from carbohydrates.

c. Acetylmethylcarbinol produced; crystalline dextrins not formed from starch .... *B. polymyxa.*

cc. Acetylmethylcarbinol not produced; crystalline dextrins formed from starch .... *B. macerans.*

bb. Gas not formed from carbohydrates.

c. Starch hydrolyzed.

d. Indole not formed; acetylmethylcarbinol not produced.

e. Growth at 65° C. .... *B. stearothermophilus.*

ee. No growth at 65° C. .... *B. circulans.*

dd. Indole formed; acetylmethylcarbinol produced .... *B. alvei.*

cc. Starch not hydrolyzed.

d. pH of glucose broth cultures less than 8.0; growth in glucose broth under anaerobic conditions.

e. Indole produced; acid from glucose and mannitol .... *B. laterosporus.*

ee. Indole not produced; no growth on the basal medium with carbohydrates .... *B. pulvifaciens.*

dd. pH of glucose broth cultures 8.0 or higher; no growth in glucose broth under anaerobic conditions .... *B. brevis.*

Group 3. Sporangia Swollen by Round Spores

aaa. Spores spherical or nearly so, terminal to subterminal; sporangia swollen; Gram-variable.
  b. Growth on ordinary mediums; growth on nutrient agar at pH 6.0.
    c. Starch hydrolyzed; growth in 10 percent NaCl broth ..... *B. pantothenticus.*
    cc. Starch not hydrolyzed; no growth in 10 percent NaCl broth .... *B. sphaericus.*
  bb. No growth on ordinary mediums; no growth at pH 6.0; urea or alkaline condition necessary for growth .... *B. pasteurii.*

DESCRIPTIONS OF SPECIES STUDIED

Group 1. Sporangia Not Definitely Swollen

Bacillus megaterium

Bacillus megaterium De Bary, Vergeleichende Morph. und Biol. der Pilze, Mycetozoen und Bakterien, p. 499. 1884.

In De Bary's report the name of this organism was spelled "megaterium." It has been assumed (*13*) that this was a typographical error and that the name was meant to be "megatherium." derived from two Greek roots, *mega* meaning great and *therium* meaning animal. Rippel (*132*), on the other hand, held that the original spelling, meaning "big rod," was the correct form. Recently, the Judicial Commission of the International Committee on Bacteriological Nomenclature of the International Association of Microbiologists (*78*) has ruled that De Bary's spelling is the proper form to be used.

In 1883 Zopf (*181, p. 66*) gave the name "*Bacterium tumescens*" (regarded here as a synonym) to a similar organism, thus antedating De Bary's report by a year. This reference is usually overlooked and Zopf's publication in 1885 (*182, p. 82*) is cited as the original. Both Zopf's and De Bary's accounts are inadequate to characterize either species, but Zopf (*182, p. 82*) compared cultures of each and regarded them as distinct. Since then, the description of *Bacillus megaterium* has often been emended and a clearer definition of the species has resulted than in the case of *B.* (*Bact.*) *tumescens.*

Criteria not listed under "characters" that have been advanced by other workers as valuable in the delineation of *B. megaterium* are as follows: According to the nutritional studies of Knight and Proom (*84*), *B. megaterium* was able to grow on ammonia basal medium, requiring no growth factors. Colmer (*34*), McGaughey and Chu (*110*), and Knight and Proom (*84*) were agreed that the egg-yolk reaction (lecithinase) of this species was negative.

The characters of *B. megaterium* listed below were based upon the study of 93 strains. Of these, 6 were type cultures, 28 bore names in synonymy, 8 had other names, 27 were received unnamed, and 24 were isolated by the writers.

CHARACTERS [9]

**Vegetative rods.*—1.2μ to 1.5μ by 2.0μ to 4.0μ; single or in short chains; ends rounded (pl. 3, *A*); stained protoplasm granular or foamy; occasional shadow-forms; motile; Gram-positive. *Variations:* [10] 0.9μ to 2.2μ by 1.0μ to 5.0μ; filaments and chains long and tangled; ends squared; photoplasm evenly stained; many shadow-forms; nonmotile; buds at end and side of rod; or Gram-variable.

On glucose-nutrient agar, the rods were larger, longer, and more vacuolated than on nutrient agar and contained numerous large fat globules (pl. 3, *B*). *Variations:* More shadow-forms than on nutrient agar; fat globules few and small; cells irregular in shape, some with pointed ends, some like corkscrews (wet mount); or Gram-variable.

**Sporangia.*—Not distinctly swollen (pl. 3, *A*).

**Spores.*—1.0μ to 1.2μ by 1.5μ to 2.0μ; oval; central or paracentral; thin-walled; many in 48 hours. *Variations:* Diameters 0.8μ to 1.4μ; shapes irregular, reniform, oviform, almost spherical, or cylindrical; lateral; or a few in 3 to 6 days.

Colonies.—Large; smooth; soft; glistening; round; convex; entire; nonspreading; dense; creamy white to yellow. *Variations:* Rough; concentrically ridged; or thin-edged.

Nutrient agar slants.—Growth abundant; smooth; soft to butyrous; opaque; glistening; slightly spreading; nonadherent; creamy white to yellow; some browning with pellucid dots on aging. *Variations:* Rough; slightly wrinkled; tough; nonspreading; or adherent.

Nutrient broth.—Turbidity medium to heavy; uniform; with or without abundant sediment; no pellicle. *Variations:* Turbidity flocculent or granular; pellicle thin and friable; or broth clear with flocculent sediment.

Glucose-nutrient agar slants.—Growth usually more abundant and softer (somewhat slimy) than on nutrient agar. *Variations:* Gummy; coarsely wrinkled; or pellucid dots more distinct than on nutrient agar.

Tyrosine agar slants.—Deep black pigment by few strains.

Utilization of citrate.—Positive.

Potato.—Growth abundant; smooth; soft to slimy; glistening; spreading; creamy white, pale to lemon yellow, or pink. *Variations:* Rough; wrinkled; potato blackened; orange-colored; or no growth.

Soybean agar slants.—Growth abundant; better sporulation and fewer shadow-forms than on nutrient agar. *Variations:* Rough; wrinkled; or sporulation slower than on nutrient agar.

**Glucose-nitrate agar slants.*—Growth very heavy; raised.

**Hydrolysis of starch.*—Positive.

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[9] The more important characters are starred (*).

[10] Variations observed are listed singly, one or more applying to each variant.

**Production of acetylmethylcarbinol.*—Negative (incubation temperature, 32° C.).

**Fermentation tests.*—Acid without gas from glucose, sucrose, and mannitol; usually acid from arabinose, xylose, and glycerol; acid variable from lactose.

Hydrolysis of gelatin.—Positive.

Hydrolysis of casein.—Positive.

Reduction of nitrate to nitrite.—Variable; majority of strains negative.

Anaerobic production of gas from nitrate.—Negative.

**Anaerobic growth in glucose broth.*—Negative.

Temperatures for growth.—Good growth at 28° to 37° C.; maximum temperature for majority of the strains 40° to 45°. *Variations:* No growth at 37°; or growth at 48°.

TYPE CULTURES STUDIED [11]

Of the nine cultures labeled *Bacillus megaterium,* six were true to type and three belonged to other species as indicated below.

Bacillus megaterium

No. 234, from AMNH, 1923, No. 734.
No. 308, from Soule, Mich. Univ., 1936.
No. 892, from Porter, Iowa Univ., 1940; Bredemann; Neide.
No. 893, from Porter, 1940; Edwards; Kral No. 19 (very slow and weak in the fermentation of carbohydrates).
No. 894, from Porter, 1940; Edwards; Conn.
No. 895, from Porter, 1940; Edwards; Novy.

From ATCC, 1939, No. 71; AMNH No. 734A; Ford. See *B. pumilus* No. 735.
From ATCC, 1939, No. 72; Ford; Kral. See *B. pumilus* No. 736.
From ATCC, 1939, No. 943; Levine No. 2062. See *B. subtilis* No. 737.

SYNONYMY

The following cultures bearing names of other supposedly distinct species were found to conform to their original descriptions and were identical with *Bacillus megaterium.*

Bacillus capri Stapp, Centbl. f. Bakt. [etc.] Abt. 2, 51: 19. 1920.

From Porter, Iowa Univ.:	*B. megaterium* No.
1937; Stapp	607
1940; Bredemann	822
1940; Claussen; Stapp	824

These three cultures agreed with each other and, in general, with Stapp's description. In all their properties they were identical with a normal *B. megaterium,* except for their reduction of nitrates to nitrites and in the slightly smaller diameter of their rods. The production of slime on glucose agar noted by Stapp was no greater than that of most strains of *B. megaterium.* Contrary to his observation that no reserve materials were present, fat was demonstrated in cells of all three strains when grown on glucose- or glycerol-nutrient agar.

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[11] Alphabetical abbreviations for the more frequent sources of cultures are as follows:

AMNH—American Museum of Natural History, New York.
ATCC—American Type Culture Collection, 2029 M St., N.W., Washington 6, D. C.
NCA—National Canners' Association, Washington, D. C.
NCTC—National Collection of Type Cultures, Lister Institute, London.
NIH—National Institutes of Health, Bethesda, Md.
USDA—United States Department of Agriculture, Washington 25, D. C.
USPHS—United States Public Health Service, Washington 25, D. C.

Bacillus carotarum Koch, Bot. Ztg. 46: [277], [325], [341], illus. 1888.

From Porter:	*B. megaterium* No.
1937; Bredemann, strain Blau	608
1940; Stapp; Claussen	828
1940; Claussen	829

The presence of cylindrical cells in long threads and single, short, swollen ones described by Koch was not unusual in a slightly rough culture of *B. megaterium.* Although Koch noted the granular appearance of the cell and the similarity in size between his bacillus and De Bary's *B. megaterium,* he considered the microscopic appearance important enough for the establishment of a new species.

From Porter, 1940; Bredemann; Stapp. See *B. circulans* No. 826.
From Porter, 1940; Stapp. See *B. pumilus* No. 827.

Bacillus cobayae Stapp, Centbl. f. Bakt. [etc.] Abt. 2, 51: 10. 1920.

From Porter:	*B. megaterium* No.
1937; Stapp	610
1940; Bredemann	835
1940; Claussen	837

The three cultures of *B. cobayae* were identical. They reduced nitrates to nitrites, as stated by Stapp, but since this was a variable character of *B. megaterium* it could not be used as a means of differentiation.

Bacillus danicus Löhnis and Westermann, Centbl. f. Bakt. [etc.] Abt. 2, 22: 253. 1908.

From Löhnis, Leipzig Univ., Germany:	*B. megaterium* No.
1914, B strain	245
1914, original strain	246

These cultures were described as capable of fixing small quantities of nitrogen in mannitol solution. This observation, however, has not been generally accepted, the explanation being that the small gains in nitrogen were due to experimental error.

From Porter, 1937; Glathe. See *B. subtilis* No. 601.

Bacillus graveolens Gottheil, Centbl. f. Bakt. [etc.] Abt. 2, 7: 496, 535. 1901.

From Porter:	*B. megaterium* No.
1937; Bredemann	615
1940; Stapp	872

Gottheil stated that the growth of *B. graveolens* was relatively thin and finely wrinkled and not so slimy or so thick as *B. tumescens* (*B. megaterium*). Lawrence and Ford (*94, p. 307*) studied a strain from Kral's collection and found that it had all the cultural reactions of *B. megaterium* with an additional tendency to produce curved or spiral forms. The writers have noticed such forms in other cultures of *B. megaterium* and have ascribed them to nutritional deficiencies. Numbers 615 and 872 did not differ from normal strains of *B. megaterium.*

From ATCC, 1939, No. 7003; Orcutt. See *B. subtilis* No. 730.

Bacillus malabarensis Löhnis and Pillai, Centbl. f. Bakt. [etc.] Abt. 2, 19: 91. 1907.

From Löhnis, 1914 .... *B. megaterium* No. 239

Although *B. malabarensis* was defined as having spindle- or wedge-shaped cells, such forms were frequently observed in cultures of *B. megaterium* and were thought to be caused by unfavorable nutrition. A bacteriophage prepared with this strain lysed 70 of 91 cultures of *B. megaterium.*

From Porter, 1940; Bredemann. See *B. pumilus* No. 620.
From Porter, 1940; Claussen. See *B. pumilis* No. 891.

Bacillus musculi Stapp, Centbl. f. Bakt. [etc.] Abt. 2, 51: 39. 1920.

From Porter:	*B. megaterium* No.
1937; Stapp	623
1940; Bredemann	907

These cultures reduced nitrates to nitrites, as stated by Stapp, but otherwise could not be distinguished from a typical *B. megaterium.* (See *B. capri* and *B. cobayae* above.)

From Porter, 1940; Claussen; Stapp. See *B. subtilis* No. 909.

Bacillus oxalaticus (Zopf) Migula, Arb. aus dem Bakt. Inst. der Tech. Hochsch. zu Karlsruhe 1: 139. 1897.

From Porter:	*B. megaterium* No.
1937; Bredemann	627
1940; Claussen	923

In 1896 Migula received an undescribed culture labeled *B. oxalaticus* from Zopf. From Migula's illustrations, showing large rods with many vacuoles, it is apparent that the original was a strain of *B. megaterium.*

From Porter, 1940; Stapp; received as *Bacillus oxalactis*, but believed to be a misspelling of *B. oxalaticus.* See *B. cereus* No. 922.

Bacillus petasites Gottheil, Centbl. f. Bakt. [etc.] Abt. 2, 7: 535. 1901.

	B. megaterium No.
From ATCC, 1937, No. 89; AMNH No. 735; Ford No. 13	343
From Porter, 1940; Stapp	931

The original description of *B. petasites* indicated a yellow strain of *B. megaterium.* Lawrence and Ford (*94, p. 305*) obtained a culture of *B. petasites* from Kral and also isolated several from soil and dust. They concluded that these cultures differed from *B. megaterium* only in their yellow pigment. Since most of the cultures of *B. megaterium* used in the present work produced some pigment varying from cream to yellow, pink, brown, or black on some medium or other, pigment formation was considered too unstable a property of this species even to characterize a variety.

From NCTC, 1937, No. 2606; Ford No. 20. See *B. subtilis* No. 338.
From Porter, 1940; Bredemann. See *B. subtilis* No. 930.
From Porter, 1940; Claussen. See *B. subtilis* No. 933.

Bacillus ruminatus Gottheil, Centbl. f. Bakt. [etc.] Abt. 2, 7: 485. 1901.

From Porter:	*B. megaterium* No.
1940; Stapp	951
1940; Claussen	952

The large diameter of the rods (1.4μ to 1.5μ), the formation of fat, and the character of the growth originally ascribed to *B. ruminatus* are characteristics of *B. megaterium.* Laubach and others (*93, p. 499*) stated that it resembled *B. megaterium* closely, except for its porcelain-white growth. Cultures Nos. 951 and 952 showed a yellow pigment on potato and agreed with *B. megaterium* in all respects.

From ATCC, 1936, No. 98; AMNH No. 793; Ford. See *B. pumilus* No. 345.
From Porter, 1937; Bredemann. See *B. subtilis* No. 634.

Bacillus silvaticus Neide, Centbl. f. Bakt. [etc.] Abt. 2, 12: 32. 1904.

From Porter:	*B. megaterium* No.
1940; Stapp	957
1940; Claussen	958

Neide stated that the rods of this species were 1.2μ to 1.6μ by 4.0μ and contained vacuoles and fat globules. The spores averaged 1.1μ by 1.7μ and before germination swelled more in width than in length. Growth on nutrient agar was creamy white, and on potato, heavy, yellowish, and slimy. Lehmann, Neumann, and Breed (*96, p. 611*) placed this organism close to *B. megaterium.* Cultures Nos. 957 and 958 were not at variance with Neide's account.

From Porter, 1937; Bredemann. See *B. subtilis* No. 636.

Bacillus (*Bacterium*) *tumescens* Zopf, Die Spaltpilze, p. 66. 1883.

	B. megaterium No.
From Porter, 1940; Stapp	991
From Porter, 1940; Edwards; Winslow	994
From Porter, 1940; Claussen	995
From ATCC, 1944, No. 4531; Ford No. 23A	1102

The history of *B. tumescens* was discussed above in connection with that of *B. megaterium.*

From Porter, 1940; Bredemann. See *B. subtilis* No. 989.
From Porter, 1940; NCTC No. 2607; Ford No. 25A. See *B. pumilus* No. 990.
From Porter, 1940; Edwards; Kral No. 23 smooth and No. 23 rough. See *B. cereus* Nos. 992 and 993.

ASSIGNMENT OF OTHER NAMED CULTURES

The cultures listed below and, as a result of these studies, assigned to *Bacillus megaterium* did not conform to or were not recognizable from their original descriptions.

Bacillus aterrimus

From Schooley, Bucknell Univ., 1936 .... *B. megaterium* No. 267

See *Bacillus subtilis* var. *aterrimus* below.

Bacillus cohaerens Gottheil, Centbl. f. Bakt. [etc.] Abt. 2, 7: 689. 1901.

From Porter, Iowa Univ.:	*B. megaterium* No.
1940; Bredemann	838B
1940; Stapp	839B

There has been considerable confusion over the identity of *B. cohaerens.* Gottheil stated that his organism was 1.1μ in diameter, of varying lengths, and, as nearly as can be judged, similar to a rough *B. megaterium.* Chester (*25, p. 84*) believed it to be identical with *B. simplex.* Lawrence and Ford (*94, p. 308*) disagreed with Chester, because the culture they obtained from Kral had rods 0.37μ to 0.65μ in diameter and spores 0.5μ to 0.56μ by 0.93μ to 1.25μ. The growth was thin, soft, spreading, whitish, becoming yellow. This same culture apparently was obtained by the writers from the NCTC and proved to be a typical *B. pumilus* (No. 334 below).

Cultures Nos. 838 and 839 of *B. cohaerens* were mixed when received. From each was obtained a small rod (*B. circulans* Nos. 838A and 839A, respectively) and a larger rod similar to *B. megaterium* (Nos. 838B and 839B, respectively). The measurements of the latter and the similarity of the two cultures to *B. simplex* confirmed Chester's observations. (See Unclassified Cultures.)

These strains were first classified by the writers (*147*) as *B. megaterium-B. cereus* intermediates because of their inability to ferment xylose and arabinose and to grow on glucose-nitrate agar. Further examination of the cultures proved that they would grow abundantly on glucose-nitrate agar after serial transfer on this medium. This finding, together with a change in the conception of an intermediate strain resulted in the reclassification of these cultures as *B. megaterium.* (See section on Rejuvenation of Aberrant Cultures.) These cultures were not typical, however, as they failed to attack the pentoses and were not sensitive to the bacteriophage.

From NCTC, 1937, No. 2596; Ford No. 10A; Kral. See *B. pumilus* No. 334.

Bacillus endoparasiticus Benedek, Mycopathologia 1: 26. 1938.

	B. megaterium No.
From ATCC, 1944, No. 7481; Benedek Type 0	1103
From Branham, NIH, 1947; Benedek No. 301–29	1224
From Branham, NIH, 1947; Benedek No. 210–35	1255

In 1927 Benedek (*12*) described an organism from skin lesions and named it *Schizosaccharomyces hominis.* Although this was later identified by certain investigators as *B. megaterium,* Benedek believed that he had a new species growing in the O, S, and R types. Of the six cultures available to the writers, three (type O) were *B. megaterium;* one (type S) was *B. cereus;* and two (type R) were *B. subtilis.*

From ATCC, 1944, No. 7480; Benedek type R. See *B. subtilis* No. 1107.
From Branham, NIH, 1948; Benedek type R. See *B. subtilis* No. 1248.
From Branham, NIH, 1948; Benedek type S. See *B. cereus* No. 1249.

Bacillus immobilis Steinhaus, Jour. Bact. 42: 783. 1941.

From Steinhaus, USPHS, 1942, No. 81........*B. megaterium* No. 1024

After moving his laboratory Steinhaus recovered this culture from a broken tube and doubted that it was the original culture. Evidently it was not, because No. 1024, a normal *B. megaterium* except for its darker pigment on tyrosine agar and potato, was entirely different from his account of *B. immobilis.*

Bacillus simplex Gottheil, Centbl. f. Bakt. [etc.] Abt. 2, 7: 685. 1901.

From Porter, 1940; Edwards; Conn ....*B. megaterium* No. 960

In spite of the emending of Gottheil's description by Lawrence and Ford (*94, p. 310*), it is not possible to recognize this species. The above strain was a normal, rough *B. megaterium.* On the other hand, four strains of *B. simplex* (Nos. 335, 346, 961, and 962) were placed as *B megaterium-B. cereus* intermediates in 1946 (*147*) mainly because of their negative characters. It is now believed that they are degenerate forms, and because of their physiological inactivity and morphological abnormality they have been placed in the list of unclassified cultures.

From ATCC, 1936, No. 4526. See *B. sphaericus* No. 349.
From Porter, 1940; Bredemann. *See B. subtilis* No. 959.

IDENTIFICATION OF UNNAMED CULTURES

The following 27 unnamed cultures were received during the course of the work and were identified as *Bacillus megaterium.*

Unnamed cultures	*B. megaterium* No.
From Meier, USDA, 1937, Nos. 13H, 13M, 15B, 16B, and 19A, from air over the Caribbean	690–694
From Reid, Pa. State Col., 1937, Nos. A1, D1, and H1, from fermenting tobacco	695–697
From Porges, Independ. Farm Labs., Ames, Iowa, 1940	771
From Steinhaus, USPHS, 1942, Nos. 16, 20, and 82, from insects (*156*)	1021, 1022, and 1025
From ATCC, 1942; Hollweg, Fort George Wright Hosp.	1026
From Townsend and Zuch (*166*), Calif. Univ. and NCA, 1943, Nos. 9, 10, 18, and 22, from incompletely sterilized materials	1071, 1072, 1080, and 1084
From Pollard, Tenn. Univ., 1946, strain A	1144
From Skinner, Minn. Univ. Med. School, 1948; McLimans Nos. SB1 and SB2	1241 and 1242
From Wheeler, La. State Univ., 1948, from interior of potato tubers	1258–1260
From Hollis, Conn. Agr. Expt. Sta., 1949, Nos. 2–4, from interior of potato tubers	1271–1273
From Benedict, USDA, 1950, No. X92	1335

Cultures Nos. 1071, 1072, 1080, and 1084 failed to ferment xylose and arabinose and grew weakly on glucose-nitrate and soybean agars. After one to five serial transfers good growth was obtained on both mediums. They were also cultivated in casein digest broth with the result that strain No. 1080 was regenerated and hydrolyzed the pentoses actively. (See section on Rejuvenation of Aberrant Cultures.)

Lutman and Wheeler (*107*), in carefully controlled experiments using special techniques, were able to demonstrate a development of typical *B. megaterium* in slices from the interior of healthy potato tubers (cultures Nos. 1258–1260). These results were later confirmed by Hollis.[12]

Culture No. 1335 was slightly aberrant because of its inability to ferment arabinose and xylose and to grow well on glucose-nitrate agar.

The writers isolated from soil and identified the following 24 strains as *B. megaterium:* Nos. 240, 241, 268–271, 283–289, 360–367, 392, 393, and 753.

Bacillus cereus

Bacillus cereus Frankland and Frankland, Roy. Soc. London, Phil. Trans., Ser. B, 173: 279. 1887.

Bacillus cereus, one of the first of this genus to be described, is widely distributed and outnumbers any other aerobic sporeformer found in the soil of the eastern United States. In spite of this, it does not appear to be well known by bacteriologists, for it has been and is still being isolated and described again and again under a host of names. The account by Frankland and Frankland, although an early one, is adequate and recognizable. A partial excuse for the bacteriologists' lack of familiarity with *B. cereus* may be the mistaken identity of *B. subtilis,* with which it was confused for some time. Conn (*36*) carefully studied the literature and concluded that the name *B. subtilis* had been applied to two different bacilli, a comparatively large-celled organism, 1.0μ or more in diameter, with large spores, and to a smaller one, 0.7μ to 0.8μ in diameter, with small spores. The former, he decided, was *B. cereus* (*B. subtilis,* Michigan strain) and the latter the true *B. subtilis* (Marburg strain).

Bacillus cereus is an excellent example of a stable "parent" species, having several varieties and variants (fig. 1). The mycoid variety (*B. mycoides*) is basically similar to *B. cereus* and upon dissociation cannot be distinguished from it. (See Studies on Dissociation.) The pathogenic variety (*B. anthracis*), which is discussed later, is also so closely related that strains which have lost their virulence cannot be separated from *B. cereus* or from dissociates of *B. cereus* var. *mycoides.* Another variety (*B. thuringiensis*) is pathogenic for insects and has the same species pattern. The writers have put more time on the study of these relationships than will appear to the reader and are confident that the arrangements shown represent a natural grouping.

In addition to the characters of *B. cereus* listed below, Colmer (*34*), McGaughey and Chu (*110*), and Knight and Proom (*84*) reported a positive egg-yolk (lecithinase) reaction for this species and its varieties. The combined results represented a total of 101 strains of *B. cereus,* 32 of var. *mycoides,* and 22 of var. *anthracis.* According to the studies of Knight and Proom on the nutrition of these organisms, *B. cereus,* in contradistinction to *B. megaterium,* was not able to grow on ammonia basal medium but required amino acids.

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[12] Personal communication, 1949.

The following description of *B. cereus* was based on 139 strains, of which 5 were type cultures, 34 bore names in synonymy, 21 carried other names, 65 were received without names, and 14 were isolations. These figures do not include 39 strains that have been assigned to the 3 varieties of this species.

CHARACTERS

**Vegetative rods.*—1.0μ to 1.2μ by 3.0μ to 5.0μ; usually in short to long tangled chains; ends square; stained protoplasm granular or foamy; no shadow-forms; not encapsulated; motile; Gram-positive. *Variations:* 0.8μ to 1.3μ by 2.0μ to 6.0μ; filaments; ends rounded; protoplasm stained uniformly; encapsulated; nonmotile; or Gram-variable.

On glucose-nutrient agar, cells were larger and more vacuolated than on nutrient agar and contained many large fat globules (pl. 3, *D*). *Variation:* Fewer, smaller fat globules but cells always vacuolated when lightly stained.

**Sporangia.*—Not appreciably swollen (pl. 3, *C*).

**Spores.*—1.0μ by 1.5μ; oval; central or paracentral; thin-walled; many in 18 to 24 hours. *Variations:* 0.5μ to 1.2μ by 1.3μ to 2.5μ; few or none at 48 hours or longer.

**Colonies.*—Large; rough; flat; irregular with whiplike outgrowths; whitish with characteristic mottled appearance by transmitted light (resembling galvanized iron or moire silk). *Variations:* Thin; spreading; very rough and arborescent; or smooth and dense.

Nutrient agar slants.—Growth abundant; rough; opaque; whitish; nonadherent; spreading; edge irregular with whiplike outgrowths. *Variations:* Relatively smooth; very rough; extending into the agar; or with greenish-yellow fluorescent pigment.

Nutrient broth.—Heavy, uniform turbidity with soft, easily dispersed sediment; with or without soft ring pellicle. *Variations:* Flocculent growth; or with firm pellicle.

Glucose-nutrient agar slants.—Growth abundant, heavier and softer than on nutrient agar.

Utilization of citrate.—Usually positive.

Potato.—Growth abundant; thick; spreading; soft; creamy-white, sometimes with pinkish tinge. *Variations:* Growth restricted; thin; folded; dry; slimy; potato darkened or orange-colored.

**Glucose-nitrate agar slants.*—Scant or no growth.

**Hydrolysis of starch.*—Positive. (Although some starch-negative cultures of *B. cereus* were described by Lamanna (*90*), the writers have found all strains positive by the method of Kellerman and McBeth (*83*). The tests included some of Lamanna's negative cultures.)

**Production of acetylmethylcarbinol.*—Positive (incubation temperature 32° C.). *Variation:* Three strains negative.

Fermentation tests.—Acid without gas from glucose; usually positive on sucrose, glycerol, and salicin; negative on arabinose, xylose, and mannitol; usually negative on lactose. (The fermentation of lactose was first considered unusual enough to warrant the separation of the positive strains into the variety *albolactis*. This plan was abandoned after several negative strains were induced to hydrolyze lactose by monthly serial transfers on lactose agar. See section on Fermentation Studies.)

Hydrolysis of gelatin.—Positive. (See pl. 2, *C*.)

Hydrolysis of casein.—Positive. (See pl. 2, *D*.)

Reduction of nitrate to nitrite.—Positive. *Variation:* Fourteen strains negative. (Conn and Breed (*38, p. 273*) reported similar observations.)

Anaerobic production of gas from nitrate.—Usually positive.

**Anaerobic growth in glucose broth.*—Positive; pH usually below 5.2.

Temperatures for growth.—Good growth at 28° to 35° C.; maximum temperature for majority of strains 45°. *Variations:* No growth at 37°; or growth at 48° C.

TYPE CULTURES STUDIED

The following authentic cultures of *Bacillus cereus* were studied.

Bacillus cereus

No. 232, from AMNH, 1923, No. 724; Ford.
No. 305, from Soule, Mich. Univ., 1936.
No. 1122, from ATCC, 1945, No. 9620; Texas Univ.
No. 1130, from Knaysi, Cornell Univ., 1945, No. C_3.
No. 1256, from Haynes, USDA, 1948, No. B–569 (penicillinase positive).

SYNONYMY

Thirty-four named cultures that apparently conformed to their original descriptions were identified as *Bacillus cereus* as follows.

Bacillus albolactis (Loeffler) Migula, Syst. der Bakt., p. 577. 1900.
B. lactis albus Loeffler, Berlin. Klin. Wchnschr. 24: 630. 1887.

	B. cereus No.
From ATCC, 1939, No. 7004	721
From Porter, Iowa Univ., 1940; NCTC No. 2601; Ford No. 15	808

Lawrence and Ford (*94, p. 287*) stated that *B. albolactis* resembled *B. cereus* except in the fermentation of lactose. Their identification was probably correct, for Migula noted that *B. albolactis* was similar to the anthrax bacillus.

Strain No. 721 fermented lactose readily. On the other hand, acid was not formed from lactose by No. 808 when it was first tested. After six monthly transfers on the basal lactose agar the culture grew moderately well and produced acid in a few days. (See section on Fermentation Studies.)

Bacillus cereus fluorescens Laubach, Jour. Bact. 1: 508. 1916.

From Porter, 1937; NCTC No. 2600; Ford No. 14 . . . *B. cereus* No. 645

This culture as originally stated was morphologically and physiologically like *B. cereus*, except that a greenish-yellow fluorescence was usually shown on nutrient agar, milk agar, and gelatin. Nitrates were not reduced to nitrites.

Bacillus ellenbachensis (Stutzer and Hartleb) Gottheil, Centbl. f. Bakt. [etc.] Abt. 2, 7: 540. 1901.
B. ellenbachensis alpha Stutzer and Hartleb, Centbl. f. Bakt. [etc.] Abt. 2, 4: 31. 1898.

From Porter:	*B. cereus* No.
1940; Bredemann; Gottheil	847
1940; Bredemann	848
1940; Stapp	849
1940; Claussen; Kläser	850
1940; Claussen	851

The writer's observations confirmed the generally accepted fact that this organism is the same as *B. cereus.*

Bacillus goniosporus Burchard, Arb. aus dem Bakt. Inst. der Tech. Hochsch. zu Karlsruhe 2: 14. 1898.

From Knaysi, Cornell Univ., 1940 .... *B. cereus* No. 767

Gottheil's (*68*) belief that this species was identical with *B. ellenbachensis* has been substantiated here.

Bacillus lacticola (Migula) Neide, Centbl. f. Bakt. [etc.] Abt. 2, 12: 168. 1904.
B. lactis V Flügge, Ztschr. f. Hyg. u. Infektionskrank. 17: 299. 1894.
Bact. lacticola Migula, Syst. der Bakt., p. 305. 1900.

	B. cereus No.
From Porter, 1937; Bredemann	617
From ATCC, 1939, No. 4342; Stewart	731
From Porter, 1940; Stapp	877

These cultures were in agreement with the morphology, macroscopic appearance, and the few physiological properties given in Neide's description.

From Porter, 1940; Claussen. See *B. pumilus* No. 878.

Bacillus lactis (Flügge) Neide, Centbl. f. Bakt. [etc.] Abt. 2, 12: 337. 1904.
B. lactis I Flügge, Ztschr. f. Hyg. u. Infektionskrank. 17: 272. 1894.

From Porter:	*B. cereus* No.
1937; Bredemann	618
1940; Bredemann; Neide	879
1940; Stapp	880
1940; Claussen	881

In 1894 Flügge (*52, p. 294*) described 12 organisms isolated from sour milk. Two years later (*53, p. 208*) he named the first one *B. lactis* I and again defined it as an actively motile, thick, short rod with terminal spores. The colonies had branchlike projections and gelatin was quickly liquefied. With the exception of the terminal spores these are characters of *B. cereus.* In 1904 Neide described *B. lactis* I more completely and the additional properties also conformed to *B. cereus.* Migula in 1900 (*115*) changed the name of Flügge's *B. lactis* I to *B. brevis.* As characterized by Ford (*93, p. 522*) in 1916 and as recognized today, cultures of *B. brevis* differ distinctly from Flügge's and Neide's organism. This situation is also discussed under *B. brevis.*

Bacillus metiens Charlton and Levine, Iowa Engin. Expt. Sta. Bul. No. 132: 18. 1937.

From Porter, 1937; Levine .... *B. cereus* No. 621

This isolation from spoiled ginger ale was first described in 1927 (*101*), then named, and more completely characterized in 1937. From the two descriptions it is recognizable as *B. cereus.*

Bacillus mycoides Flügge, Die Mikroorganismen, p. 324. 1886.

From Waksman, N. J. Agr. Expt. Sta., 1948, No. 7A. . *B. cereus* No. 1266

This culture did not show the typical rhizoid growth of *B. mycoides* while under the writers' observation. It is possible, of course, that it did at one time and subsequently dissociated to its present form. (See Studies on Dissociation.)

Bacillus petroselini Burchard, Arb. aus dem Bakt. Inst. der Tech. Hochsch. zu Karlsruhe 2: 39. 1898.

From Porter, 1940; Claussen .... *B. cereus* No. 934

This species has rightly been believed to be identical with *B. cereus.*

Bacillus praussnitzii Trevisan, I Generi e Spec. d. Batteriacee, p. 20. 1889.

From Porter, 1940; NCTC No. 2603; Ford No. 17...... *B. cereus* No. 935

Culture No. 935 was not rhizoid nor did it ferment lactose, both properties of authentic strains. It may be a dissociant, however, and for further discussion, see the synonymy of *B. cereus* var. *mycoides* and the section on Studies on Dissociation.

Bacillus robur Neide, Centbl. f. Bakt. [etc.] Abt. 2, 12: 18. 1904.

From Porter:	*B. cereus* No.
1940; Stapp	946
1940; Claussen	947

These are probably authentic cultures because Neide noted the close similarity of this species to *B. cereus* and *B. cursor.* Lehmann, Neumann, and Breed (*96, p. 604*) regarded *B. robur* as closely related to, if not identical with, *B. cereus.* Cultures Nos. 946 and 947 failed to form nitrites from nitrates, as did certain other strains of this species (*38*).

From Porter, 1937; Bredemann. See *B. subtilis* No. 631.

Bacillus sessilis Klein, Centbl. f. Bakt. [etc.] 6: 349, [377]. 1889.

From Knaysi, 1940 .... *B. cereus* No. 768

This organism was found by Klein in the blood of a supposed case of bovine anthrax a few hours after the death of the animal. The cells appeared singly, occasionally in chains; some were motile; others nonmotile. Although the isolate resembled *B. anthracis,* injection of guinea pigs and cattle caused no reaction. (See *B. siamensis* and *B. tropicus* below.)

Bacillus siamensis Siribaed, Jour. Infect. Dis. 57: 142. 1935.

From Siribaed, Natl. Health Lab., Siam, 1935 ...... *B. cereus* No. 201

Siribaed isolated this strain from a blood culture and reported that it was highly pathogenic to laboratory animals. Two years later Clark (*28*) found it to be nonvirulent and identical with *B. cereus.* A bacteriophage prepared with this strain lysed all cultures of *B. cereus, B. anthracis,* and the nonrhizoid dissociants of *B. mycoides.*

Bacillus subtilis, Mich. strain, Conn, Jour. Infect. Dis. 46: 341. 1930.

	B. cereus No.
From Soule, Mich. Univ., 1936	303
From NIH, 1937, No. 19	537
From NIH, 1937, No. 60; Evans; Conn; Pribram	538
From Porter, 1940; Edwards; Soule	970
From Porter, 1940; Edwards; Novy	976
From Porter, 1940; Edwards; Conn	973
From Porter, 1940; Edwards; Conn; Chester	974

The first five strains, some of which very likely are duplicates, did not reduce nitrates to nitrites, whereas the last two strains were positive in this respect.

Bacillus tropicus Heaslip, Med. Jour. Austral. 28: 536. 1941.

From Heaslip, Med. and Vet. Sci. Inst., Adelaide, South Australia, 1941 .... *B. cereus* No. 793

Heaslip obtained this organism from mice that had been inoculated with the blood of human patients. Its cultural characteristics were not distinguishable from those of *B. anthracis*. It was pathogenic for mice, guinea pigs, and rabbits and caused a fever in man. Serologically, it was related to *B. anthracis*, and Heaslip admitted the possibility of its being a variant of that species. Strain No. 793 was an actively motile, normal *B. cereus*. Tests made in 1943 for pathogenicity by the Pathological Laboratory, Bureau of Animal Industry, United States Department of Agriculture, were negative. These results were comparable with Clark's findings on *B. siamensis* (*28*).

Bacillus undulatus Dooren de Jong, Zentbl. f. Bakt. [etc.] Originale (1) 122: 277. 1931.

From Porter:	*B. cereus* No.
1940; Dooren de Jong No. 1110	998
1940; Dooren de Jong No. 1102	999

The original description of this organism is clearly one of *B. cereus*. Later, Dooren de Jong (*46*) dissociated *B. mycoides* in peptone water and reported that the stable S form was the same as *B. undulatus*. He failed, however, to see the similarity of his dissociants to *B. cereus*. (See Studies on Dissociation.)

ASSIGNMENT OF OTHER NAMED CULTURES

The 21 cultures listed below did not conform to their original descriptions or were not recognizable because of the inadequacy of their characterizations. They were studied in detail and identified as *Bacillus cereus* as follows.

Bacillus aethylicus Fitz, Ber. der Chem. Gesell. 6: 48. 1873; 9: 1348. 1876; 10: 276. 1877.
B. fitzianus Zopf, Die Spaltpilze, p. 52. 1883.

From Porter, Iowa Univ., 1940; Pribram .... *B. cereus* No. 804

From the various accounts of this species it was not possible to judge whether or not culture No. 804 was authentic.

Bacillus agri Laubach and Rice, Jour. Bact. 1: 516. 1916.

From ATCC:	*B. cereus* No.
1936, No. 4507; Ford No. 12	310
1936, No. 2; AMNH No. 800; Ford	342

These cultures did not agree with a duplicate of Ford's No. 12 obtained through the NCTC. (See immediately below.) The original account of *B. agri* is too indefinite to determine whether the ATCC or the NCTC strain is true to type.

From NCTC, 1937, No. 2598: Ford No. 12. See *B. pumilus* No. 336.
From Lamanna, Camp Detrick, 1947, No. 13. See *B. brevis* No. 1219.

Bacillus aterrimus

From ATCC:	*B. cereus* No.
1939, No. 944; Levine No. 2025	722
1939, No. 4509; Ford No. 5	723

These two cultures did not form the black pigment characteristic of *B. aterrimus*, nor did they agree with it in other respects. (See *B. subtilis* var. *aterrimus* below.) Except for slightly smaller than normal vegetative rods they were identical with a typical *B. cereus*.

Bacillus coagulans

From Porter, 1940; Bact. Dept., Iowa State Col. ...... *B. cereus* No. 833

For authentic cultures, see *B. coagulans* below.

Bacillus endoparasiticus Benedek, Mycopathologia 1: 26. 1938.

From Branham, NIH, 1948; Benedek type S .... *B. cereus* No. 1249

Three other cultures bearing this name and originally from Benedek have been assigned to *B. megaterium* (Nos. 1103, 1224, and 1225) and two to *B. subtilis* (Nos. 1107 and 1248).

Bacillus fusiformis

From Porter, 1940; Claussen .... *B. cereus* No. 867

See *B. sphaericus* in group 3.

Bacillus hollandicus

From Porter, 1940; Claussen .... *B. cereus* No. 875

See *B. brevis* in group 2.

Bacillus lactimorbus

From ATCC, 1939, No. 246; Jordan .... *B. cereus* No. 733

See strain No. 732 in the synonymy of *B. sphaericus* (group 3).

Bacillus lactis niger

	B. cereus No.
From Gorini, Milan, Italy, 1936, No. 1A	256
From Cameron, NCA, 1937; Breed; Gorini No. 1A	654

These duplicates did not form the black pigment specified by Gorini. On the other hand, his strain No. 2 of *B. lactis niger* blackened mediums containing tyrosine. (See *B. subtilis* var. *niger* Nos. 254 and 655.) Numbers 256 and 654 fermented lactose readily, two of the few cultures of *B. cereus* that did.

Bacillus lasiocampa Brown, Amer. Mus. Novitates No. 251: 7. 1927.

From ATCC, 1944, No. 4415 .... *B. cereus* No. 1092

For cultures associated with insects, see *B. cereus* var. *thuringiensis*.

Bacillus laterosporus

From NIH, 1937, Clinical Pathogen Series .... *B. cereus* No. 589

See *B. laterosporus* in group 2.

Bacillus luteus (Smith and Baker) Garbowski, Centbl. f. Bakt. [etc.] Abt. 2, 19: [641], [731]. 1907; 20: 99. 1907.

From Porter, 1940; Claussen .... *B. cereus* No. 886

Smith and Baker (*148*) stated that this organism resembled *B. mesentericus fuscus* of Flügge in many respects, but that it was larger and formed a bright-yellow pigment. Lehmann, Neumann, and Breed (*96, p. 611*) classified it under *B. megaterium*. In addition to the above, another strain studied by the writers proved to be *B. subtilis* (No. 619) and still another *B. pumilus* (No. 885). None of these strains formed a yellow pigment. Lacking pertinent data on the original culture, it was not possible to determine the true identity of *B. luteus*.

Bacillus mesentericus ruber

From Porter, 1937; Torrey Res. Sta., England .... *B. cereus* No. 644

See the synonymy of *B. licheniformis* below.

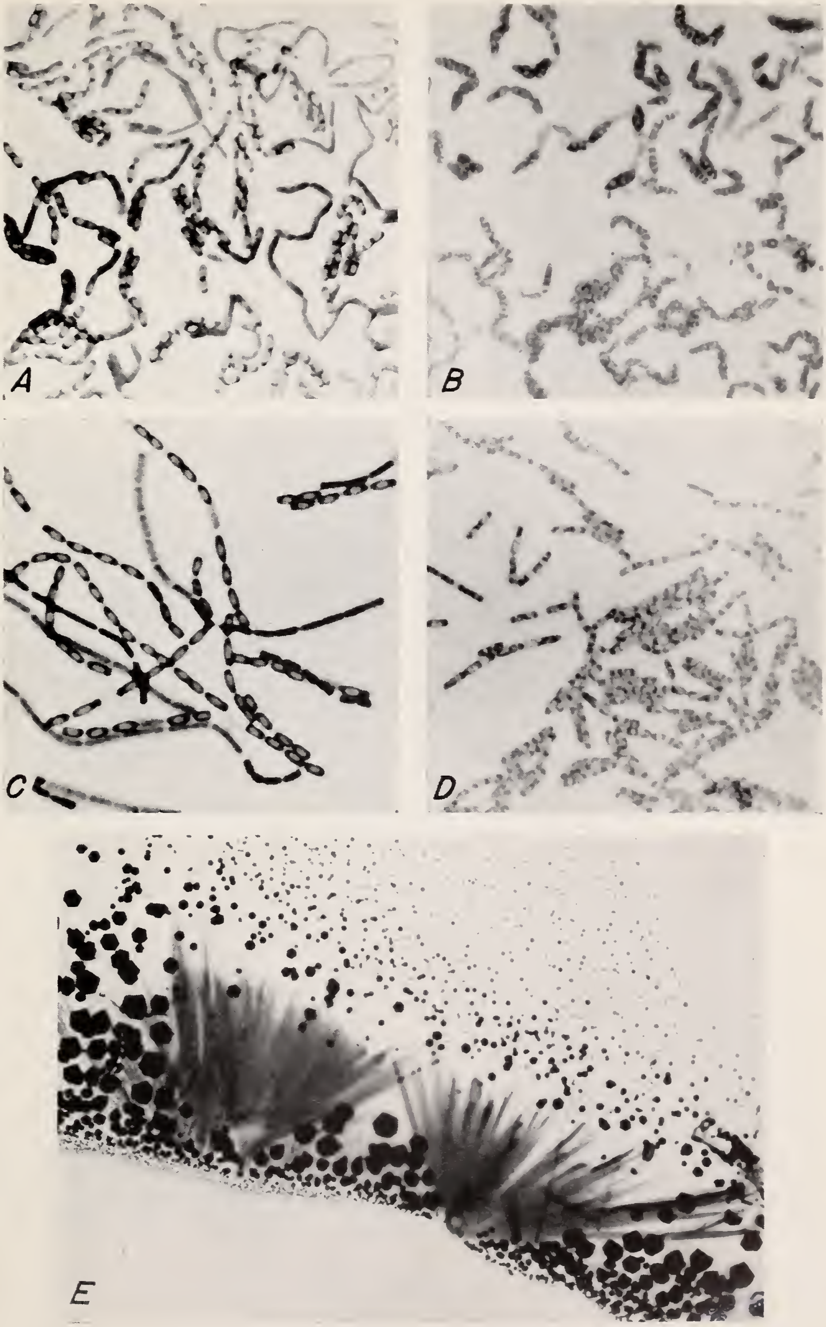
Bacillus mesentericus viscosus

From Porter, 1940; Torrey Res. Sta., England ...... *B. cereus* No. 903

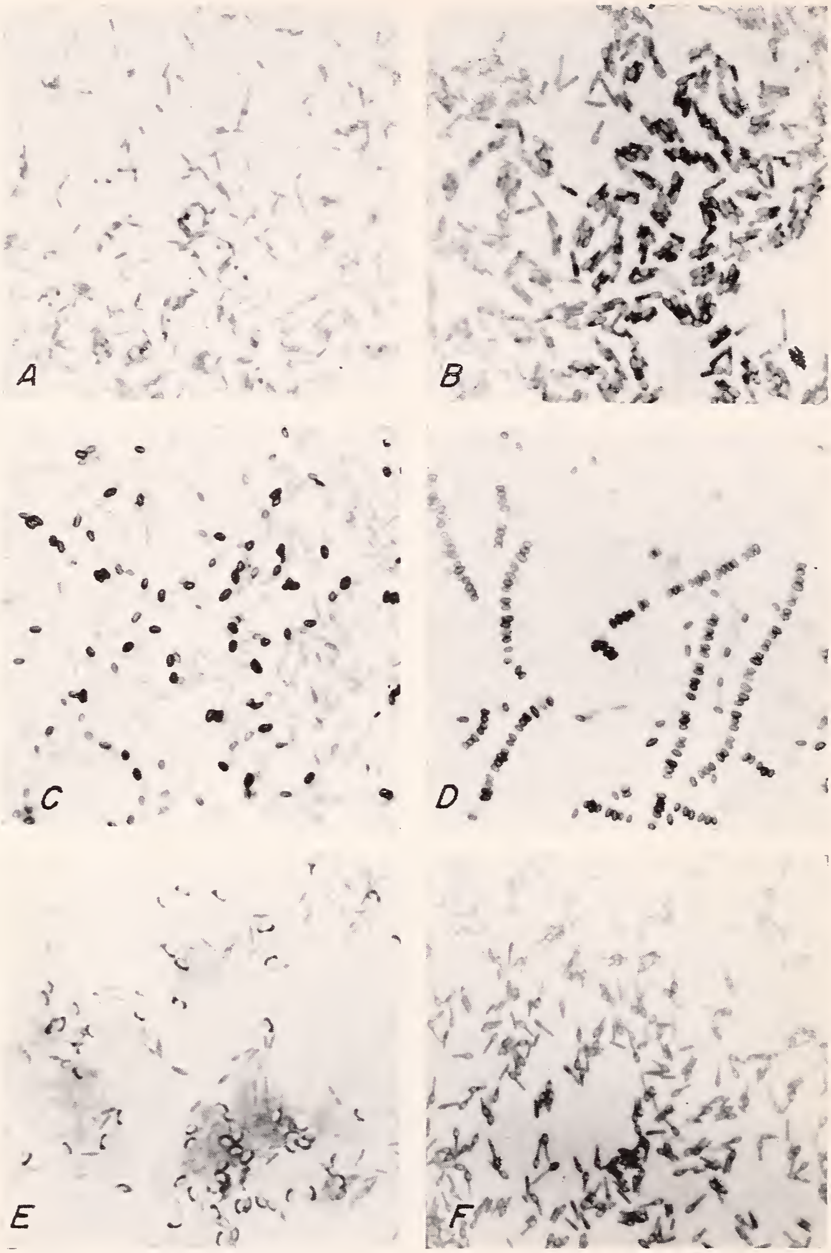
This culture did not correspond to *B. mesentericus panis viscosus* I or II. For a discussion of the latter, see *B. panis* in the synonymy of *B. subtilis*.

Bacillus oxalactis

From Porter, 1940; Stapp .... *B. cereus* No. 922



Morphology of *Bacillus megaterium: A*, on nutrient agar after 1 day; *B*, on glucose-nutrient agar after 3 days. Morphology of *B. cereus: C*, on nutrient agar after 1 day; *D*, on glucose-nutrient agar after 3 days. *E*, Crystalline dextrins produced by *B. macerans*. (*A* to *D*, approximately × 1,000; *E*, approximately × 100.)



Typical morphology of certain species: *A*, Sporangia of *Bacillus subtilis,* showing bipolar staining; *B*, sporangia of *B. stearothermophilus; C*, spores of *B. circulans; D*, spores of *B. alvei* lying parallel in rows; *E*, remnants of sporangia adhering to spores of *B. laterosporus; F*, sporangia of *B. sphaericus.* (Approximately × 1,000; stained by aqueous fuchsin.)

This name has not been found in the literature and is probably a misspelling of *B. oxalaticus* Zopf. See the synonymy of *B. megaterium.* Lehmann, Neumann, and Breed (*96, p. 611*) reported that the diameter of the rods of a culture of *B. oxalaticus* obtained from Kral was smaller than that given by Miglua (*114*) and that other characters resembled those of the anthrax bacillus. Strain No. 922 is probably of the same origin.

Bacillus pumilus

From Porter, 1940; Claussen .... *B. cereus,* No. 942

This culture bore no resemblance to *B. pumilus* as described below.

Bacillus tumescens

From Porter:	*B. cereus* No.
1940; Edwards; Kral No. 23 smooth	992
1940; Edwards; Kral No. 23 rough	993

Authentic cultures under this name (Nos. 991, 994, 995, and 1102) may be found in the synonymy of *B. megaterium.*

IDENTIFICATION OF UNNAMED CULTURES

The following unnamed cultures were studied and identified as *Bacillus cereus.*

Unnamed cultures	*B. cereus* No.
From Herron, Queen's Univ., Canada, 1930	235 and 248
From NIH, 1937, Nos. 8, 10, 14, 16, 29, 35, 41, 66, 77, and 80	401–410
From NIH, 1937, No. 46	411
From NIH, 1937, Nos. 47–50, 137, 143, and 182	539–545
From NIH, 1937, from spinal fluid	599
From Babers, USDA, 1937	600
From Haffkine Inst., Bombay, India, 1938	701
From Randall, Georgetown Univ. Med. School, 1939, from blood culture	720
From Mollari, Georgetown Univ. Med. School, 1941, from puncture wound	785
From Mollari, 1941, from blood culture	786
From Ross, Reid Memorial Hosp., Richmond, Ind., 1941, from spinal fluid	794
From Allen, Tenn. Univ., 1942, from rayon bath	1006
From Steinhaus, USPHS, 1943, Nos. 1D1, 1E1, 1H1, 1I1, 2A1, 2D1, 2F1, 2G1, 2H1, 9A1, 17E1, 17F1, 17H1, 17I1, 20C1, 20D1, 21A1, 21B1, 21C1, 21D1, 21E1, 21L1, 22B2, and 96–A, from *Amblyomma americanum*	1036–1059
From Bohrer, NCA, 1944, Nos. M18 and M38	T42 and T44
From Steinhaus, 1945; Sokoloff and Klotz, *Bacillus "C"*	1129
From Chance, Okla. Univ., 1946	1140
From Brown, Ariz. Agr. Expt. Sta., 1946, strain "B"	1142
From Reuszer, USDA, 1946	1216
From Schneiter, NIH, 1947, No. 2, from horsehair autoclaved for 15 minutes at 121° C.	1231
From Schneiter, 1947, No. 2L, from lesion in mouse injected with No. 1231	1232
From Schneiter, 1947, No. MB73, from heart blood of a mouse inoculated with culture from hair exposed to methyl bromide	1236
From Clancy, Pa. Hosp., Philadelphia, 1948, strain ARO, from infection following mastectomy	1261
From Haydak, Minn. Univ., 1949, from ventriculus of queen bee	1274
From Delaporte, Lab. de Cytologie Végétal, Paris, 1949, No. 9P3	1309
From Perkins, Hawaiian Pineapple Co., Honolulu, 1950	1332

Culture No. 248 conformed to the species pattern of *B. cereus* in all respects except in its fermentation of xylose and fair growth on glucose-nitrate agar. In 1946 (*147*) it was placed as an intermediate between *B. megaterium* and *B. cereus* because of these unusual reactions. Later observations, however, proved that its growth on glucose-nitrate agar could not be increased by serial transfer to approximate the abundant growth of *B. megaterium* and that it correlated with *B. cereus* in its production of high acidity in glucose broth under anaerobic conditions. On the basis of this information the writers have preferred to regard culture No. 248 as a xylose-positive biotype of *B. cereus* rather than as an intermediate strain.

Because of its negative V-P reaction, culture No. 411 was formerly (*147*) regarded as a *B. megaterium-B. cereus* intermediate. It is now considered to be merely a weakened strain of *B. cereus*.

The seven cultures Nos. 539–545 did not produce nitrites from nitrates.

Babers (*4*) obtained organism No. 600 from septicemia of the southern armyworm. Because of its negative V-P reaction it was formerly (*147*) placed with the *B. megaterium-B. cereus* intermediates. It is now, however, recognized as merely a degenerate strain of *B. cereus*.

When culture No. 1140 was received it did not form spores and has not been induced to do so. Large, globular, heavily stained bodies, however, were observed. In other respects the culture was typical of *B. cereus*.

Culture No. 1216, along with Nos. 411 and 600, failed to form acetylmethylcarbinol.

The three cultures from Schneiter (Nos. 1231, 1232, 1236) were nonmotile.

When first tested culture No. 1261 did not produce acetoin; a year later, however, a positive reaction was obtained by cultivating the culture on potato prior to its inoculation in V-P broth. (See section on Rejuvenation of Aberrant Cultures.)

Number 1332 utilized xylose and was weak in the hydrolysis of starch. It may, therefore, be considered as a biotype along with No. 248 above.

The following 14 cultures were isolated by the writers and were identified as *B. cereus:* Nos. 202–210, 244, 249, and 368–370. These were obtained from soil or air with the exception of No. 202, which came from a blood culture.

Bacillus cereus var. *mycoides*

Bacillus cereus var *mycoides*. Smith, Gordon, and Clark, U. S. Dept. Agr. Misc. Pub. 559: 54. 1946.
B. mycoides Flügge, Die Mikroorganismen, p. 324. 1886.

Physiologically and in the size and appearance of its cells and spores, *Bacillus mycoides* proved to be identical with *B. cereus*. In growth characteristics, however, it differed remarkably, growing on solid mediums in long twisted strands, curving and spreading thinly over the surface. In the section Studies on Dissociation, it has been shown that this character was lost by growing the rhizoid cultures in 100 ml. of nutrient broth, plating after a few days, and selecting nonrhizoid colonies. (See pl. 1, *A*.) The resulting cultures, which could not be distinguished from *B. cereus*, remained stable, reversion to the mycoid state not taking place. *Bacillus mycoides* has, therefore, been designated as a variety of *B. cereus* rather than as a separate species.

The above conclusion was based on a study of 5 type cultures, 1 that bore a name in synonymy, 1 received unnamed, and 15 isolations.

TYPE CULTURES STUDIED

The following authentic strains were studied and designated as *Bacillus cereus* var. *mycoides*.

Bacillus mycoides

No. 233, from AMNH, 1923; Ford and Lawrence.

No. 306, from Soule, Mich. Univ., 1936.
No. 911, from Porter, Iowa Univ., 1940; Edwards; Conn.
No. 912, from Porter, 1940; Claussen.
No. 1316, from Delaporte, Lab. Cytol. Vég., Paris, 1949.

From Porter, 1940; Bredemann; Gottheil. See *B. brevis* No. 910.
From Waksman, N. J. Agr. Expt. Sta., 1948, No. 7A. See *B. cereus* No. 1266.

SYNONYMY

The following named strain conformed to its original description and was identified as *Bacillus cereus* var. *mycoides.*

Bacillus praussnitzii Trevisan, I Generi e Spec. d Batteriacee, p. 20. 1889.
B. ramosus liquefaciens Flügge, Die Mikroorganismen, p. 324. 1886.

From Porter, Iowa Univ., 1940; Edwards;
AMNH .... *B. cereus* var. *mycoides* No. 936

An organism was isolated by Praussnitz in Flügge's laboratory and described by Flügge in 1886 as *B. ramosus liquefaciens* because of its rapid liquefaction of gelatin and the bristly edge of the colonies. It was named *B. praussnitzii* in 1889 by Trevisan. Laubach (*93, p. 495*) stated that *B. praussnitzii* resembled *B. mycoides* in morphology and growth appearance but differed in its active fermentation of lactose. It also liquefied gelatin rapidly, but he did not use this character to separate it from *B. mycoides.* Only one mycoid culture fermenting lactose was received, and one (No. 320) was isolated from soil. Both of these when grown in 100 ml. of nutrient broth for several days dissociated just as *B. mycoides* had done. *Bacillus praussnitzii,* therefore, might be considered either as a rhizoid stage of *B. albolactis,* the lactose-positive variant of *B. cereus,* or as a lactose-positive biotype of *B. mycoides* (fig. 1).

From Porter, 1940; NCTC No. 2603; Ford No. 17. See *B. cereus* No. 935.

IDENTIFICATION OF UNNAMED CULTURES

The following unnamed culture was identified as *B. cereus* var. *mycoides.*

Unnamed culture

From Reuszer, USDA, 1946 ....*B. cereus* var. *mycoides* No. 1215

This strain was the only one of var. *mycoides* that did not form acetoin.

Fifteen additional strains were isolated from soil and air (Nos. 273, 317–328, 371, and 372).

Bacillus cereus var. *anthracis*

Bacillus cereus var. *anthracis.* Smith, Gordon, and Clark, U.S. Dept. Agr. Misc. Pub. 559: 55. 1946.
B. anthracis Cohn emend. Koch, Beitr. z. Biol. der Pflanz., Heft 2, 2: 279. 1876.
B. anthracis Cohn, Beitr. z. Biol. der Pflanz., Heft 2, 1: 177. 1872.

The literature on *Bacillus anthracis* is extensive; it will not be presented here because it is outside the scope of the present work. It is in the province of the writers, however, to call the attention of pathologists to the very close relationship existing between the anthrax bacillus and *B. cereus.* Stein (*155*) stated, "Pathogenicity constitutes the principal point of difference between typical strains of *B. anthracis* and those of anthrax-like organisms." In the absence of tests for pathogenicity, he found the following characters of material assistance, when considered as a whole: Motility, reduction of methylene blue, hemolysis, fermentation of salicin, rapidity of gelatin liquefaction, action on litmus milk, and colony characteristics. These tests, however, were used in the negative sense. For example, an isolation that was motile or produced marked acidity in salicin broth was not *B. anthracis*. In the writers' collection there were, however, several nonmotile strains of *B. cereus*, particularly the nonrhizoid dissociants of the variety *mycoides*, and several strains that failed to ferment salicin. Although the hemolytic activity of these strains was not studied, Bekker (*10, 11*) reported that it was a variable character and did not correlate with virulence.

Fourteen cultures labeled *B. anthracis*, two of which (Nos. 1007 and 1014) were known to be virulent, were examined in the same manner as those of *B. cereus*. None of the characters investigated separated the cultures of *B. anthracis* from those of *B. cereus*. In other words, the species pattern of *B. anthracis* was the same as that of *B. cereus*.

The loss of pathogenicity by cultures of *B. anthracis* seems to be a generally accepted phenomenon. Of the many references in the literature the most recent are those of Chu (*27*) and Tomcsik (*164, 165*). Following Sterne's technique (*159*), Chu easily obtained avirulent dissociants from cultures of *B. anthracis*. Tomcsik, continuing the experiments of Manninger and Nogradi (*111*), developed smooth strains in which delayed capsule formation was preceded by motility.

It is readily admitted that much work needs to be done by someone having facilities for animal inoculation, with the following question always in mind: When a strain of *B. anthracis* loses its pathogenicity is it still the anthrax bacillus or is it *B. cereus?* From the incomplete work of the writers and from the natural relationships of *B. cereus* (fig. 1), it would seem that the latter represents the true status of the nonvirulent strain and that from the academic viewpoint *B. anthracis* is taxonomically a pathogenic variety of *B. cereus*.

TYPE CULTURES STUDIED

The following cultures of *Bacillus anthracis* were studied and are now designated as *B. cereus* var. *anthracis*.

Bacillus anthracis

No. 1007, from ATCC, 1941, No. 10: NIH.
No. 1008, from ATCC, 1941, No. 240; Jordan; NIH.
No. 1009, from ATCC, 1941, No. 241; Jordan; NIH.
No. 1010, from ATCC, 1941, No. 937; Eichorn.
No. 1011, from ATCC, 1941, No. 938; Eichorn.
No. 1012, from ATCC, 1941, No. 4229; Bur. Animal Indus., USDA.
No. 1013, from ATCC, 1941, No. 4728; Bur. Animal Indus., USDA.
No. 1014, from ATCC, 1941, No. 6605; Army Med. School.
No. 1015, from ATCC, 1941, No. 6606; Army Med. School.
No. 1016, from ATCC, 1941, No. 6602; Army Med. School.
No. 1017, from ATCC, 1941, No. 6603; Army Med. School.
No. 1018, from ATCC, 1941, No. 6604; Army Med. School.
No. 1019, from ATCC, 1941, No. 6607; Army Med. School.
No. 1020, from Tilley, USDA, 1935.

Bacillus cereus var. *thuringiensis*

Bacillus cereus var. *thuringiensis*, new combination.
B. thuringiensis Berliner, Ztschr. f. Angew. Ent., Beihefte 2: 29. 1915.

Berliner isolated this organism from the larvae of the flour moth (*Ephestia kuehniella* Zell.). Twelve years later Mattes (*112*) studied the same organism, especially cytologically, but spelled the name *Bacillus thuringensis* both in the text and in the citation of Berliner's work. Leaving out the letter "i" was no doubt unintentional. Excluding pathogenicity, these two descriptions and a later one by Chorine (*26, p. 50*) apply to *B. cereus* except for the statement by Chorine that mannitol was fermented, a character absent in *B. cereus* and in the strains of *B. thuringiensis* available for study.

In 1946 (*147*) the one strain of Mattes' organism at hand seemed to conform to the species pattern of *B. cereus*, and *B. thuringiensis* was, therefore, placed in the synonymy of *B. cereus*.

Recently Steinhaus (*157*) reopened the question of the pathogenicity of the sporeformers for insects and found Mattes' original strain still virulent. A reexamination of smears of Mattes' culture showed a morphological characteristic in the tendency of the spore to lie obliquely in the sporangium, leaving two knobs of protoplasm after the sporangium disintegrated. This feature, which is illustrated in Mattes' drawings, together with pathogenicity for insects, has not been observed in cultures of *B. cereus* (*157*).

Only three strains pathogenic for insects were examined; all were identical with *B. cereus* except for the distinctive appearance of their sporangia. Until more information can be gathered it has been thought best to place *B. thuringiensis* tentatively as a variety of *B. cereus* rather than as a separate species. This is a purely academic view of the relationship and is in line with the treatment given the anthrax organism above.

TYPE CULTURE STUDIED

The following culture was classified as *Bacillus cereus* var. *thuringiensis*.

Bacillus thuringiensis

No. 996, from Porter, Iowa Univ., 1940; Mattes.

IDENTIFICATION OF UNNAMED CULTURES

Two isolates reported (*157*) pathogenic for insects were received for study.

Unnamed cultures

From Steinhaus, Calif. Univ., 1945, from Indian-meal moth (*Plodia interpunctella* (Hbn.) .... *B. cereus* var. *thuringiensis* No. 1124
From Steinhaus, 1950, No. B13, from larva of moth (*Aphomia gularis* Zeller) .... *B. cereus* var. *thuringiensis* No. 1328

Unlike strain No. 996, Nos. 1124 and 1328 did not form acetylmethylcarbinol.

Bacillus licheniformis

Bacillus licheniformis (Weigmann) Gibson, Soc. Agr. Bact. Abs. Proc. 1937; Jour. Dairy Res. 13: 248. 1944.

Clostridium licheniforme Weigmann, Centbl. f. Bakt. [etc.] Abt. 2, 4: 820. 1898.

The elevation of Ford's strain of *Bacillus subtilis* to species rank, i.e., *B. licheniformis,* by Gibson will be discussed below under *B. subtilis.* Of the many characteristics of *B. subtilis* and *B. licheniformis* investigated by the writers only the latter's ability to grow well anaerobically, forming high acidity in glucose broth and gas in alkaline nitrate broth, divided the two species. At the same time, however, it should be recognized that so far there is no proof that the oxygen relationships of one or the other may not be subject to variation. If *B. licheniformis,* after dissociation, failed to grow anaerobically and to produce acid from glucose or gas from nitrate, the writers would then be in the embarrassing position of having recognized a species capable of changing into another. There may be, however, other equally important lines of separation not yet observed by them. Lamanna (*91*) has presented evidence, based on a relatively few strains, that the spore antigens of the two species may be different.

The nutritional requirements (*84*) and the egg-yolk (lecithinase) reaction of *B. licheniformis* (*34, 110*) have been found to be the same as for *B. subtilis.* Gibson (*63*) reported that the ciliate or tangled outgrowths developed at the edge of the growth on agar were generally diagnostic. The writers, however, failed to find this character of definite value when applied to a number of strains of *B. subtilis* and *B. licheniformis.* It is hoped, therefore, that future study will bring to light additional evidence by which the two species may be differentiated or placed more closely together.

The following description of *B. licheniformis* was based on an examination of 63 strains, of which 3 were type cultures, 16 carried names in synonymy, 8 bore other names, 35 were unnamed, and 1 was isolated by the writers.

CHARACTERS

**Vegetative rods.*—0.6μ to 0.8μ by 1.5μ to 3.0μ; not in chains; stain uniformly; no capsules or shadow-forms; motile; Gram-positive. *Variations:* 0.5μ to 0.9μ by 1.2μ to long filaments; encapsulated; shadow-forms; or nonmotile.

On glucose-nutrient agar the rods were the same as on nutrient agar and contained a few fat globules. *Variations:* Larger cells; filaments; encapsulated; or Gram-variable.

**Sporangia.*—Little, if any, bulging.

**Spores.*—0.6μ to 0.9μ by 1.0μ to 1.5μ; oval to cylindrical; central or paracentral; thin-walled; many in 48 hours at 37° C. *Variations:* 0.5μ to 1.0μ by 1.0μ to 2.0μ; or few in 2 to 3 weeks.

Colonies.—Rough or rugose surface; hairy outgrowths; spreading; offwhite. *Variations:* Smooth; entire; or lobate.

**Nutrient agar slants.*—Growth abundant; rough; opaque; adherent; spreading; matt surface; hairy outgrowths. *Variations:* Smooth; rugose; thin; or nonadherent.

Nutrient broth.—Clear with heavy, wrinkled, tough pellicle. *Variations:* Flocculent or uniform turbidity; with or without fragile pellicle.

NaCl broth.—Good growth in 5 to 8 percent NaCl; inhibition with higher concentrations; in a few cases, growth in 12 percent.

**Glucose-nutrient agar slants.*—Growth heavy; rugose; often extruded droplets or mucoid vesicles; usually hairy outgrowths from line of inoculation; sometimes gas formation at 37° C.

Tyrosine agar slants.—Growth same as on nutrient agar.

**Utilization of citrate.*—Positive.

**Potato.*—Growth heavy; spreading; wrinkled; warty; droplets extruded; pink; red to violet on submerged portion of potato. *Variations:* Soft; or offwhite.

**Soybean agar slants.*—Growth softer and more abundant than on nutrient agar; brownish to reddish, often purplish at the bottom; droplets extruded. *Variations:* Matt surface; or folded.

Glucose-nitrate agar slants.—Growth slow; scant to abundant; offwhite to brownish red; hairy outgrowth; sometimes gas formed.

**Hydrolysis of starch.*—Positive.

**Production of acetylmethylcarbinol.*—Positive (37° C. better than 32°).

**pH of glucose broth.*—Variable, pH 5.2 to 8.2 at 7 days.

**Fermentation tests.*—Acid without gas from arabinose, xylose, glucose, sucrose, and mannitol; acid variable from lactose (at 14 days).

**Hydrolysis of gelatin.*—Positive; wide zone of hydrolysis.

**Hydrolysis of casein.*—Positive; usually narrow zone of clearing.

**Reduction of nitrate to nitrite.*—Positive.

**Anaerobic production of gas from nitrate.*—Positive.

**Anaerobic growth in glucose broth.*—Positive; pH 5.2 or lower at 14 days; usually small amount of gas.

**Temperatures for growth.*—Good growth 32° to 45° C.; maximum temperature between 50° and 56°.

TYPE CULTURES STUDIED

The following three strains of *Bacillus licheniformis* were received and examined.

Bacillus licheniformis

Nos. 1263–1265, from Gibson, Edinburgh Univ., Scotland, 1948, Nos. 5, 46, and 601.

SYNONYMY

The following 16 named cultures were identified as *Bacillus licheniformis.*

Bacillus globigii Migula, Syst. der Bakt., p. 554. 1900.

B. mesentericus ruber Kruse in Flügge, Die Mikroorganismen, p. 199. 1896.

Red potato bacillus, Globig, Ztschr. f. Hyg. u. Infektionskrank. 3: 322. 1888.

From Delaporte, Lab. Cytol. Vég., Paris, 1949, Nos. 2K7, 3A2 blanc, 3A12 jaune, and 8A2 .... *B. licheniformis* Nos. 1289–1292

Although the early descriptions are meager and the identity of the original strain very much in doubt, the listing of *B. globigii* in the synonymy of *B. licheniformis* by Cunningham (*41*) and Gibson (*63*) has been accepted by the writers.

From ATCC, 1936, No. 4516; Ford No. 7; Kral. See *B. circulans* No. 313.
From NCTC, 1937, No. 2593; Ford No. 7; Kral. See *B. pumilus* No. 331.
From Bacon Labs., Watertown, Mass., 1947, Red strain; Phillips; McCoy. See *B. subtilis* var. *niger* No. 1221A.
From Bacon Labs., 1947, Brown strain; Phillips. See *B. subtilis* var. *niger* No. 1221B.

Bacillus mesentericus ruber (Globig) Kruse in Flügge, Die Mikroorganismen, p. 199. 1896.

Red potato bacillus, Globig, Ztschr. f. Hyg. u. Infektionskrank. 3: 322. 1888.

From Porter, Iowa Univ., 1940 .... *B. licheniformis* No. 900

Although the early accounts are of little value in deciding the identity of the original culture, Gibson's listing of *B. mesentericus ruber* in the synonymy of *B. licheniformis* (*63*) has been followed by the writers.

From Porter, 1937; Torrey Res. Sta. See *B. cereus* No. 644.

Bacillus subtilis, Ford strain, Gibson, Jour. Dairy Res. 13: 248. 1944.

	B. licheniformis No.
From AMNH, 1923, No. 723; Ford	243
From Soule, Mich. Univ., 1936; Ford	304
From NIH, 1937, No. 3	560
From ATCC, 1939, No. 6598; Army Med. School	745
From Porter, 1940; NCTC No. 2586; Ford No. 1	978
From ATCC, 1945, No. 8480; Lamanna No. 88; Soule	1128
From Knaysi, Cornell Univ., 1950, Nos. 43–45, 58, and 79	1323–1327

ASSIGNMENT OF OTHER NAMED CULTURES

The authenticity of the following cultures, identified as *Bacillus licheniformis*, could not be established.

Bacillus abysseus ZoBell and Upham, Scripps Inst. of Oceanography Bul. 5: 273. 1944.

From ZoBell, Scripps Inst., 1945, No. 620 ... *B. licheniformis* No. 1114

In contradistinction to the published account of the species, culture No. 1114 had an optimum temperature of 28° to 40° C.; acid was produced from glycerol, xylose, and lactose; and growth was as heavy in fresh water as in NaCl broth.

Bacillus calidolactis Hussong and Hammer, Jour. Bact. 15: 186. 1928.

From Porter, Iowa Univ., 1940; NCTC No. 2690 .. *B. licheniformis* No. 821

This culture did not agree with the original strain, which was described as having an optimum growth temperature of 55° to 65° C.

From Curran, USDA, 1944, Unnumbered and No. 11. See *B. stearothermophilus* Nos. T15 and T16.
From Brooks, N. Y. [Geneval Agr. Expt. Sta., 1947; Iowa State Col., Nos. C1, O1, and sp. See *B. coagulans* Nos. T2006–T2008.

Bacillus filiformis (Duclaux) Tils, Ztschr. f. Hyg. u. Infektionskrank. 9: 294. 1890.

Tyrothrix fiiliformis Duclaux, Le Lait. p. 249. 1887.

From Dubos, Rockefeller Inst. Med. Res., 1940;
NCTC No. 2120 .... *B. licheniformis* No. 778

As it is not possible to recognize Duclaux' culture from his description or illustrations, no conclusion could be drawn as to the authenticity of No. 778.

Bacillus niger

From Porter, 1940; Fabian; Gorini .... *B. licheniformis* No. 913

See *B. subtilis* var. *niger*.

Bacillus nitroxus Beijerinck, Centbl. f. Bakt. [etc.] Abt. 2, 25: 49. 1909.

From Porter, 1940; Kluyver .... *B. licheniformis* No. 918

Beijerinck described his culture as exhibiting rods, cocci, yeast forms, and clostridia, depending on the medium used. Strain No. 918 was quite different from his account.

Bacillus serrulatus Stührk, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 193. 1935.

From Porter, 1940; Bredemann .... *B. licheniformis* No. 955

Culture No. 955 bore no resemblance to Stührk's report of his organism, which apparently belonged to the *B. circulans* complex.

Bacillus tenuis (Duclaux) Migula, Syst. der Bakt., p. 587. 1900.
Tyrothrix tenuis Duclaux, Le Lait, p. 218. 1894.

From Dubos, Rockefeller Inst. Med. Res., 1940;
NCTC No. 2118, var. A .... *B. licheniformis* No. 776

Variety B of this organism, obtained from the same source as variety A, proved to be *B. subtilis*, agreeing with the generally accepted identity of this species.

From Dubos, Rockefeller Inst. Med. Res., 1940; NCTC No. 2119, var. B. See *B. subtilis* No. 777.

Bacillus vulgatus

From NCTC, 1927, No. 1097; Ling .... *B. licheniformis* No. 329

See the synonymy of *B. subtilis*.

IDENTIFICATION OF UNNAMED CULTURES

Thirty-five unnamed strains assembled during the progress of this work were identified as *Bacillus licheniformis*.

Unnamed cultures	*B. licheniformis* No.
From NIH, 1937, Nos. 21, 57, and 58	551, 557, and 558
From Reid, Pa. State Col., 1937, Nos. B2 and E11	669 and 700
From Myers, Nat'l Dairy Labs., Baltimore, 1939, No. RB.13A, from ropy bread	766
From Bur. Animal Indus., USDA, 1943, No. 379	1031
From Townsend and Zuch (*166*), Calif. Univ. and NCA, 1943, Nos. C, 15, 16, 17, 20, 24, and 26	1062, 1077–1079, 1082, 1086, and 1087
From Doudoroff, Calif. Univ., 1945	1123
From Daily, Eli Lilly and Co., Indianapolis, 1947, No. X-36	1217
From Schneiter, NIH, 1947, Nos. 3HB, MB2, and MB73	1233–1235
From Casas-Campillo, N. J. Agr. Expt. Sta., 1949	1269
From Bohrer, NCA, 1947, No. C-1960	T2001
From Olsen, Sugar Refinery Lab., Copenhagen, 1949, Nos. S5, N15, H3, H6, St 2, M4, G3, N2, G15, and B4a	T2028–T2036 and T2040
From Food and Drug Adm., 1950, Nos. BT1 and BT3, bacitracin strains (*80*)	1330 and 1331
From Benedict, USDA, 1950, No. B–1001, bacitracin strain	1333
From Stanier, Calif. Univ., 1951, No. 10	1363

One isolation made from flour was identified as *B. licheniformis* No. 712.

Bacillus subtilis

Bacillus subtilis Cohn, emend. Prazmowski, Untersuchungen über die Entwickl. und Ferment. einigen Bakterien Arten, Inaug. Diss., Leipzig. 1880.

B. subtilis Cohn, Beitr. z. Biol. der Pflanz., Heft 2, 1: 174; Heft 3, 1: 188. 1875; Heft 2, 2: 249. 1876.

There has been a great deal of confusion regarding the identity of *Bacillus subtilis,* largely because of the inadequate early descriptions and the distribution under that name of cultures that were in reality the commonly occurring *B. cereus.* After a careful study Conn (*36*) came to the conclusion, which was later accepted by the International Committee on Bacteriological Nomenclature (*137*), that the so-called Marburg strain of *B. subtilis* fitted the early descriptions.

Nothing was known about the various stages of growth that a species may exhibit—the smooth, rough, mucoid, and dwarf—until well after the turn of the century. In 1903, Chester (*25*) concluded that *B. subtilis* and *B. vulgatus* (Flügge) Trevisan (*167*) were identical, because the cultural characters upon which their separation depended were too variable. His leadership was not followed by Lawrence and Ford (*94, p. 292*), who stated that the two species could be separated by "careful observation of the cultural reactions, particularly in broth and on potato." Gibson (*62, 63*) recognized that *B. vulgatus* was the same as the Marburg strain of *B. subtilis.* He considered the so-called Ford strain of *B. subtilis*, however, different from the Marburg strain and named it *B. licheniformis* (Weigmann) in recognition of the priority of Weigmann's *Clostridium licheniforme* (*176*).

Lamanna (*89, 91*) divided his cultures of *B. subtilis* and *B. vulgatus* into two groups on the basis of the spore antigen and the equatorial germination of the spores (with or without splitting along the transverse axis). He recommended that the Ford strain be taken as the type species of the genus and that the Marburg strains be called *B. vulgatus.* Thus both Gibson and Lamanna agreed in the recognition of two distinct species but differed in the selection of the one to be used as the type of the genus.

In 1946 (*147*) before Gibson's publications were seen, the writers placed the Marburg and Ford strains together as *B. subtilis* because the characters studied did not furnish a basis for their separation. In view of the work cited above and their own more recent investigations they have accepted Gibson's designations of the Marburg strain as *B. subtilis* and the Ford strain as *B. licheniformis.*

The following information along lines not investigated by the writers has been supplied by other workers: Of the 27 strains of *B. subtilis* studied by Knight and Proom (*84*) 26 grew with ammonia as the sole source of nitrogen and needed no growth factors. The egg-yolk (lecithinase) reaction has been reported as negative (*34, 84, 110*).

The following description of _B. subtilis_ was based on a study of 156 strains of which 17 were type cultures, 23 bore names in synonymy, 34 carried other names, 80 were unnamed, and 2 were isolations. These figures do not include 45 strains belonging to the 2 black-pigmented varieties of this species.

CHARACTERS

*_Vegetative rods._—0.7μ to 0.8μ by 2.0μ to 3.0μ; not in chains; ends rounded; uniformly stained; no capsules; no shadow-forms; motile; Gram-positive. _Variations:_ 0.6μ to 1.0μ by 1.3μ to 6.0μ to filaments; long chains; encapsulated; shadow-forms; a very few strains Gram-variable; a few nonmotile; or budding from end of rod.

On glucose-nutrient agar, rods were the same as on nutrient agar and contained a few small fat globules. _Variations:_ Larger cells; more filaments and longer chains; encapsulated; or Gram-variable.

*_Sporangia._—Very little, if any, swelling by spores. (Those with young spores often resembled bipolar stained rods. See pl. 4, _A_.)

*_Spores._—0.6μ to 0.9μ by 1.0μ to 1.5μ; oval to cylindrical; central or paracentral; thin-walled; many in 48 hours. _Variations:_ 0.5μ to 1.0μ by 1.0μ to 2.0μ; or few in 15 to 21 days.

Colonies.—Rough; dull; opaque; spreading; offwhite. _Variations:_ Smooth to slimy; soft; thin; translucent; dendroid; yellow; orange; or brown.

Nutrient agar slants.—Growth abundant; rough; opaque; dull; waxy; spreading; cream-colored to light brown. _Variations:_ Smooth; slimy; thin; translucent; dendroid; yellow; or orange. (Some strains showed a greenish-yellow fluorescence when incubated at 45° C.)

Nutrient broth.—Clear with heavy, wrinkled, waxy, tough pellicle. _Variations:_ Flocculent or uniform turbidity; with or without fragile pellicle.

*_NaCl broth._—Good growth in 5-percent concentration of NaCl; growth at 7-percent (except No. 714) ; growth in a few cases at 10- or 12-percent.

*_Glucose-nutrient agar slants._—Growth heavier and softer than on nutrient agar; sometimes pink or brown.

*_Tyrosine agar slants._—Growth same as on nutrient agar.

*_Utilization of citrate._—Positive. _Variation:_ Two strains negative.

*_Potato._—Growth heavy; wrinkled to coarsely folded; spreading; offwhite, yellow, pink, or brown. _Variations:_ Slimy; soft; thin; or warty.

*_Soybean agar slants._—Growth more abundant and softer than on nutrient agar.

Glucose-nitrate agar slants.—Abundant growth. _Variation:_ Scant growth by seven strains.

Glucose-asparagine agar slants.—Abundant growth. *Variation:* Scant growth by six strains.

**Hydrolysis of starch.*—Positive (pl. 2, *A* and *B*).

**Production of acetylmethylcarbinol.*—Positive (37° C. better incubation temperature than 32°). *Variation:* One strain negative.

**pH of glucose broth.*—pH 5.0 to 8.6 at 7 days. *Variation:* Two strains with lower pH.

**Fermentation tests.*—Acid without gas from arabinose, xylose, glucose, sucrose, and mannitol; usually negative on lactose.

**Hydrolysis of gelatin.*—Positive; wide zone of hydrolysis.

Hydrolysis of casein.—Positive; usually wide zone of hydrolysis.

**Reduction of nitrate to nitrite.*—Positive. (Three originally negative cultures were induced to reduce nitrates. See section on Rejuvenation of Aberrant Cultures.)

**Anaerobic production of gas from nitrate.*—Negative.

**Anaerobic growth in glucose broth.*—Scant, if any, growth; pH 5.5 or higher at 14 days.

Temperatures for growth.—Good growth at 28° to 40° C.; maximum temperature for majority of strains 50°. *Variations:* No growth at 40°; or growth at 55°.

TYPE CULTURES STUDIED

The following 17 authentic cultures of *Bacillus subtilis* were received.

Bacillus subtilis

No. 231, from Kellerman, USDA, 1912 (used by Jansen and Hirschmann (*79*) for production of subtilin).
No. 743, from ATCC, 1939, No. 465; Harris (acetylmethylcarbinol production very weak and erratic).
No. 744, from ATCC, 1939, No. 6051; Conn, Marburg strain.
No. 968, from Porter, Iowa Univ., 1940; Bredemann; Gottheil (greenish-yellow fluorescent pigment on nutrient agar at 45° C. and a dark-pink, almost purple growth on potato at 28°).
No. 969, from Porter, 1940; Stapp.
No. 971, from Porter, 1940; Bact. Dept., Iowa State Col.; Soule (greenish-yellow fluorescent pigment on nutrient agar at 45° C.).
No. 972, from Porter, 1940; Edwards; Marburg Univ., Cohn strain (same pigmentation as No. 968 above).
No. 975, from Porter, 1940; Edwards; Meyer, Cohn strain.
One unnumbered strain discarded, from Blumberg, Columbia Univ., 1941.
No. 1088, from Raper, USDA, 1943, No. B-558 (assay of streptomycin and penicillin).
No. 1109, from ATCC, 1944, No. 9524; Merck and Co. (assay of penicillin, streptomycin, and streptothricin).
No. 1125, from ATCC, 1945, No. 9799; NCTC No. 6276 (penicillinase positive).
No. 1126, from Waksman, N. J. Agr. Expt. Sta., 1945, No. ST2.
No. 1286, from Delaporte, Lab. Cytol. Vég., Paris, 1949, No. S1 brilliant.
No. 1287, from Delaporte, 1949, No. S1 mat.
No. 1288, from Delaporte, 1949, No. 13R1.
No. 1315, from NCTC, 1950, No. 3610, Marburg strain.

From AMNH, 1923, No. 723; Ford. See *B. licheniformis* No. 243.
From Soule, Mich. Univ., 1936; Ford. See *B. cereus* No. 303.
From Soule, 1936; Ford. See *B. licheniformis* No. 304.
From NIH, 1937, No. 19. See *B. cereus* No. 537.
From NIH, 1937, No. 60; Evans; Conn; Pribram. See *B. cereus* No. 538.
From NIH, 1937, No. 3. See *B. licheniformis* No. 560.

From ATCC, 1939, No. 6598; Army Med. School. See *B. licheniformis* No. 745.
From Porter, 1940; Edwards; Soule. See *B. cereus* No. 970.
From Porter, 1940; Edwards; Conn. See *B. cereus* No. 973.
From Porter, 1940; Edwards; Conn, Chester strain. See *B. cereus* No. 974.
From Porter, 1940; Edwards; Novy. See *B. cereus* No. 976.
From Porter, 1940; NCTC No. 2586; Ford No. 1. See *B. licheniformis* No. 978.
From ATCC, 1945, No. 8480; Lamanna No. 88; Soule. See *B. licheniformis* No. 1128.
From Delaporte, 1949, Lister, hay, and Marburg strains. See *B. pumilus* Nos. 1299–1301.
From Knaysi, Cornell Univ., 1950, Nos. 43–45, 58, and 79. See *B. licheniformis* Nos. 1323–1327.

SYNONYMY

The following 23 cultures, which bore names other than *B. subtilis* and apparently conformed to their original descriptions, were identified as *Bacillus subtilis*.

Bacillus aterrimus

	B. subtilis No.
From Hall, Colo. Univ. Med. School, 1936, No. 798B; Ford	263
From NCTC, 1937, No. 2590; Ford No. 5A	330
From Cameron, NCA, 1937; Breed; NCTC No. 2590; Ford No. 5A	652
From Porter, Iowa Univ., 1940; NCTC No. 2590; Ford No. 5A	814

Number 263 failed to reduce nitrates in the routine test. After four serial transfers at weekly intervals in nitrate broth, nitrites were positive, becoming strong in the tenth serial culture. None of these cultures formed the characteristic black pigment of *B. aterrimus* on potato or carbohydrate mediums. As it has been shown (Studies on Dissociation) that the loss of this pigmentation could be brought about, it seemed likely that these were colorless dissociants of the originals. This view was strengthened by the fact that Ford's strain No. 5B (*B. subtilis* var. *aterrimus* No. 653), which was probably separated from No. 5A, still produced the black pigment.

Bacillus lactis niger

From Gorini, Milan, Italy, 1936, No. 1B	*B. subtilis* No. 255

For a discussion of this species, see *B. subtilis* var. *niger* below.

Bacillus mesentericus

From Blumberg, Columbia Univ., 1941	*B. subtilis* (discarded)

Bacillus mesentericus fuscus Flügge, Die Mikroorganismen, p. 321. 1886.

From Porter:	*B. subtilis* No.
1940; Bredemann	897
1940; Edwards; Marburg Univ.; Cohn	898

For a discussion of *B. mesentericus* and *B. mesentericus fuscus*, see *B. pumilus*.

Bacillus mesentericus hydrolyticus Herman and Neuschul, Biochem. Ztschr. 281: 219. 1935.

From Porter, 1940; Herman	*B. subtilis* No. 901

This isolation from carrots was reported as having a strong hydrolytic action on sugar, soluble starch, and the connective tissue of carrots, peas, cucumbers, radishes, and other vegetables. Culture No. 901 failed to exhibit any special hydrolytic ability. Another identical strain was received under the name of *B. vulgatus hydrolyticus*. (See below.)

Bacillus natto Sawamura, Tokyo Nogyo Diagaku (Tokyo Agr. Col. Bul.) 7: 108. 1906. See also Tokyo Nogyo Daigaku (Tokyo Agr. Col. Jour.) 5: 789. 1913.

From Hanzawa, Imperial Univ., Tokyo:	*B. subtilis* No.
1930	351
1932	352

The characteristic flavor of natto (fermented soybeans) is reputed due to this organism. Numbers 351 and 352 were typical *B. subtilis* cultures.

Bacillus panis Migula, Syst. der Bakt., p. 576. 1900.
B. mesentericus panis viscosus II Vogel, Ztschr. f. Hyg. u. Infektionskrank. 26: 404. 1897.

	B. subtilis No.
From ATCC, 1936, No. 82; AMNH No. 796; Ford No. 5	315
From NCTC, 1937, No. 2594; Ford No. 8	332
From Porter, 1940; NCTC No. 2594; Ford No. 8	926

There has been a great deal of confusion, caused by the inadequate early descriptions, regarding the so-called slimy bread organism. Laubach (*93, p. 501*) identified an encapsulated isolate as *B. panis.* After continued cultivation on artificial mediums it lost its capsules, although it remained rather slimy. Lehmann, Neumann, and Breed (*96, p. 615*), in discussing bacilli from slimy bread state, "Many 'species' of spore-forming bacteria are described which . . . are interrelated and also more or less closely related to *B. mesentericus* and *B. vulgatus.*"

The duplicate cultures Nos. 332 and 926 were not encapsulated, and no slime was evident in carbohydrate mediums. The growth of No. 315 was extremely rough and slow at 28° C.; there was no growth at 40°. Its fermentation of carbohydrates was weak and no acetylmethylcarbinol was formed. Inoculation into glucose broth and serial transfer in other mediums failed to develop a smooth or more active strain.

From ATCC, 1936, No. 4522; Ford No. 8. See *B. pumilus* No. 316.

Bacillus parvus Neide, Centbl. f. Bakt. [etc.] Abt. 2, 12: 344. 1904.

From ATCC, 1944, No. 4944; NCTC .... *B. subtilis* No. 1106

Neide reported that his organism hydrolyzed starch and placed *Tyrothrix tenuis* (*B. subtilis*) in the synonymy with it. Culture No. 1106 did not grow well on the basal carbohydrate mediums.

From Porter, 1937; Bredemann. See *B. pumilus* No. 629.
From Porter, 1940; Claussen. See *B. pumilus* No. 928.

Bacillus subtilis var. *viscosus* Chester, Del. Agr. Expt. Sta. Ann. Rpt. (1903): 84. 1904.

From Porter, 1940; NCTC No. 2587; Ford No. 2...... *B. subtilis* No. 979

Chester obtained a culture marked *B. subtilis* from Kral and another labeled *B. mesentericus-fuscus* from Johns Hopkins University and found them similar to *B. subtilis,* except for their mucoid and viscid growth. He wrote, "This feature is so strongly developed as to make it worthy of special note." Strain No. 979 formed a soft smooth growth in the present study, but it was not slimy. Its action on carbohydrates was somewhat slower than that of rough strains of the species.

Bacillus tenuis (Duclaux) Migula, Syst. der Bakt., p. 587. 1900.
Tyrothrix tenuis Duclaux, Le Lait, p. 218. 1894.

From Dubos, Rockefeller Inst. Med. Res., 1940; NCTC No. 2119, var. B. .... *B. subtilis* No. 777

Chester (*24, p. 273*) observed that the growth of this organism was similar to that of *B. vulgatus* on potato and like *B. subtilis* on agar, in milk, and in broth. Lehmann, Neumann, and Breed (*96, p. 608*) classified it between *B. subtilis* and *B. vulgatus* and Gibson (*63*) listed it in the synonymy of *B. subtilis.*

From Dubos, Rockefeller Inst. Med. Res., 1940; NCTC No. 2118, var. A. See *B. licheniformis* No. 776.

Bacillus vulgatus (Flügge) Trevisan, I Generi e Spec. d. Batteriacee, p. 19. 1889.
B. mesentericus vulgatus Flügge, Die Mikroorganismen, p. 322. 1896.

	B. subtilis No.
From Scales, USDA, 1922	237
From AMNH, 1923, No. 725; Ford	238
From Ark, Calif. Univ., 1941	787
From Porter, 1940	1003
From Porter, 1940; NCTC No. 2588; Ford No. 3	1004

A statement of the relationship of *B. vulgatus* to *B. subtilis* will be found in the discussion of *B. subtilis*. Of the cultures listed above, two possessed the characteristic folded, pigmented growth formerly associated with *B. vulgatus*, whereas the other three had the finely wrinkled, rough growth of *B. subtilis*.

From NCTC, 1927, No. 1097; Ling. See *B. licheniformis* No. 329.

Bacillus vulgatus hydrolyticus

From ATCC, 1939, No. 6894; Hermann.............. *B. subtilis* No. 747

This strain was originally named *B. mesentericus hydrolyticus* (see above) and was found to be the same as No. 901. The change in name cannot be explained.

ASSIGNMENT OF OTHER NAMED CULTURES

The 34 cultures listed below did not conform to, or were not recognizable from, their original descriptions and have been identified by the writers as *Bacillus subtilis*.

Bacillus adhaerens Stührk, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 183. 1935.

From Porter, Iowa Univ., 1940; Bredemann........ *B. subtilis* No. 802

Stührk's characterization of this strain, isolated from the soil of a banana plantation, would seem to place it in the *B. circulans* group. Culture No. 802 was evidently not the original. Furthermore, the name of this organism is a homonym, because Laubach (*93, p. 503*) had previously described under the same name a species which he said slightly resembled *B. mycoides*.

From ATCC, 1936, No. 1; AMNH; Ford. See *B. pumilus* No. 309.
From NCTC, 1937, No. 2604; Ford No. 18. See *B. pumilus* No. 337.

Bacillus borborokoites ZoBell and Upham, Scripps Inst. Oceanography Bul. 5: 239. 1944.

From ZoBell, Scripps Inst., 1945, No. 625............ *B. subtilis* No. 1115

Culture No. 1115, a typical *B. subtilis*, differed from ZoBell and Upham's account in the following respects: Optimum temperature 28° to 40° C. instead of 20° to 25°; acid from glycerol, xylose, and sucrose; strictly aerobic instead of facultative; and heavy growth in fresh-water broth instead of a fair growth. The culture was inhibited by a 10-percent concentration of NaCl and did not grow in 12-percent. Several other strains of *B. subtilis* from nonhalophilic sources possessed the same salt tolerance.

Bacillus butyricus Hueppe, Mitt. aus dem K. Gesundheitsamte 2: 353. 1884.

From Porter, 1940; NCTC No. 1650; Kral.......... *B. subtilis* No. 820

After investigations of a culture maintained in stock for a long time Lehmann, Neumann, and Breed (*96, p. 610*) stated that this organism stood between *B. megaterium* and *B. mesentericus* and was in some respects like *B. vulgatus*. Number 820 exhibited a fairly smooth rather than a typical rough growth and was slightly slower and weaker on certain mediums than most strains of *B. subtilis*.

Bacillus danicus

From Porter, 1937; Glathe .... *B. subtilis* No. 601

See synonymy of *B. megaterium* Nos. 245 and 246.

Bacillus endoparasiticus Benedek, Mycopathologia 1: 26. 1938.

	B. subtilis No.
From ATCC, 1944, No. 7480; Benedek type R	1107
From Branham, NIH, 1948; Benedek type R	1248

Three other cultures bearing this name from Benedek have been assigned to *B. megaterium* (Nos. 1103, 1224, and 1225) and one to *B. cereus* (No. 1249).

Bacillus geniculatus De Bary, Beitr. zur Kenntnis der niederen Organismen in Mageninhalt, Inaug. Diss., Strassburg and Leipzig. 1885.

From Dubos, Rockefeller Inst. Med. Res., 1940; NCTC No. 2123 ... *B. subtilis* No. 772

Migula (*115, p. 525*) noted no difference between this organism and *B. subtilis* except in the smaller diameter of the rods (0.5μ to 0.6μ). Lehmann, Neumann, and Breed (*96, p. 608*) placed it as a synonym of *B. parvus*. They also listed (*96, p. 616*) *B. geniculatus* Duclaux (*Tyrothrix geniculata* Duclaux) (*50, p. 231*) as a relative of *B. panis*, the "slimy-bread" bacillus.

Bacillus graveolens

From ATCC, 1939, No. 7003; Orcutt ... *B. subtilis* No. 730

Authentic cultures are included in the synonymy of *B. megaterium*. Number 730 was one of the few cultures of *B. subtilis* that grew in a 12-percent concentration of NaCl.

Bacillus luteus

From Porter, 1937; Bredemann; Garbowski ... *B. subtilis* No. 619

See list of other named species assigned to *B. cereus*.

Bacillus megaterium

	B. subtilis No.
From ATCC, 1939, No. 943; Levine No. 2062	737
From Blumberg, Columbia Univ., 1941	Discarded

For authentic cultures, see *B. megaterium*, the first species described above.

Bacillus (*Tyrothrix*) *minimus* Duclaux, Le Lait, p. 213. 1887.

From Dubos, 1940; NCTC No. 2122; Rosenthal; Duclaux ... *B. subtilis* No. 773

No discussion of the possible relation of this organism to others has been found in the literature.

Bacillus musculi

From Porter, 1940; Claussen; Stapp ... *B. subtilis* No. 909

See list of synonyms of *B. megaterium*.

Bacillus orae Werner, Zentbl. f. Bakt. [etc.] Abt. 2, 87: 464. 1933.

From Porter, 1940; Bredemann ... *B. subtilis* No. 919

The original account seems to indicate that this organism belonged to the *B. circulans* complex.

Bacillus petasites

	B. subtilis No.
From NCTC, 1937, No. 2606; Ford No. 20	338
From Porter, 1940; Bredemann	930
From Porter, 1940; Claussen	933

See synonymy of *B. megaterium*.

Bacillus pumilus

	B. subtilis No.
From ATCC, 1939, No. 4344	741
From Porter, 1940; Claussen	941

These cultures reduced nitrates to nitrites, hydrolyzed starch, and were in the rough stage. See description of *B. pumilus* below.

Bacillus robur

From Porter, 1937; Bredemann . . . . *B. subtilis* No. 631

See synonymy of *B. cereus.*

Bacillus rugulosus Stührk, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 181. 1935.

From Porter, 1940; Bredemann . . . . *B. subtilis* No. 949

Stührk reported that this organism did not form nitrites from nitrates, although its growth on potato resembled that of *B. subtilis.* Other characters given by him did not help in determining the authenticity of No. 949.

Bacillus ruminatus

From Porter, 1937; Bredemann . . . . *B. subtilis* No. 634

See synonymy of *B. megaterium.*

Bacillus scaber (Duclaux) Migula, Syst. der Bakt., p. 586. 1900.
Tyrothrix scaber Duclaux, Ann. Inst. Natl. Agron. 4: 94. 1882.

From Dubos:	*B. subtilis* No.
1940; NCTC No. 2116, var. A	774
1940; NCTC No. 2117, var. B	775

The writers have not been able to obtain the original description by Duclaux. In a later work (*50, p. 236*) he stated that the granular vegetative rods were 1.1μ to 1.2μ in diameter and his illustrations showed a resemblance to *B. cereus.* This evidence would lead to the belief that Nos. 774 and 775 were not authentic.

Bacillus silvaticus

From Porter, 1937; Bredemann . . . . *B. subtilis* No. 636

See synonymy of *B. megaterium.*

Bacillus simplex

From Porter, 1940; Bredemann . . . . *B. subtilis* No. 959

See assigned strains under *B. megaterium.*

Bacillus sphaericus

From Porter, 1940; Bredemann . . . . *B. subtilis* No. 963

See *B. sphaericus* in group 3.

Bacillus submarinus ZoBell and Upham, Scripps Inst. Oceanography Bul. 5: 267. 1944.

From ZoBell, 1945, No. 569 . . . . *B. subtilis* No. 1120

Culture No. 1120, a typical *B. subtilis,* was at variance with its original account in the following respects: Optimum temperature 28° to 40° C. instead of 20° to 25°; acid from xylose; strictly aerobic instead of facultative; and heavy growth in fresh-water broth instead of a fair growth. The culture was inhibited by a 10-percent concentration of NaCl and did not grow in 12-percent. Several other strains of *B. subtilis* from nonhalophilic sources possessed the same salt tolerance.

Bacillus tardivus Stührk, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 177. 1935.

From Porter, 1940; Bredemann . . . . *B. subtilis* No. 981

The original organism apparently belonged to the round-spored group (possibly *B. sphaericus*) and was quite different from the present culture.

Bacillus teres Neide, Centbl. f. Bakt. [etc.] Abt. 2, 12: 161. 1904.

From Porter, 1940; Claussen ....*B. subtilis* No. 987

None of the three cultures bearing this name could be assigned to the same species, and no definite information is available to determine whether any one of them is authentic. See cultures assigned to *B. pumilus* and list of unclassified cultures.

Bacillus terminalis

From Porter, 1940; NCTC No. 2612; Ford No. 28....*B. subtilis* No. 983

Ford's strain No. 28 obtained from another source proved to be *B. circulans* (No. 746), which also did not conform to the original description of the species.

Bacillus thalassokoites ZoBell and Upham, Scripps Inst. Oceanography Bul. 5: 268. 1944.

From ZoBell, 1945, No. 576.......................*B. subtilis* No. 1121

Culture No. 1121 did not agree with the following characters given in the above reference: Optimum temperature was 28° to 40° C. rather than 20° to 25°; the growth was strictly aerobic instead of facultative, and heavy rather than fair in fresh-water broth. A 9-percent concentration of NaCl was inhibitive; scant growth occurred in 10-percent; and none in 12-percent. Number 1121 was not, therefore, more salt-tolerant than several other strains of *B. subtilis.*

Bacillus tumescens

From Porter, 1940; Bredemann ....*B. subtilis* No. 989

See synonymy of *B. megaterium.*

Bacillus watzmannii Werner, Zentbl. f. Bakt. [etc.] Abt. 2, 87: 462. 1935.

From Porter, 1937; Bredemann ....*B. subtilis* No. 642

The spores of this organism were described as having a variety of shapes and sizes and the sporangia as club- or spindle-shaped. The original strain, therefore, probably belonged to the *B. circulans* complex.

IDENTIFICATION OF UNNAMED CULTURES

The following 80 unnamed cultures, studied during the progress of this work, were found to be typical for *Bacillus subtilis.*

Unnamed cultures	*B. subtilis* No.
From NIH, 1937, Nos. 30–32, 38, 40, 37, 54, and 115	552–556, 559, 579, and 581
From Reid, Pa. State Col., 1937, No. Cl	698
From Cordon, N. J. Agr. Expt. Sta., 1938	702
From Presley, USDA, 1938	703
From Curran, USDA, 1938	705
From Cohen, Harvard Univ., 1938	714
From Myers, Natl. Dairy Labs., Baltimore, 1939, Nos. RBKr. 5. RBKr. 5–Y, and RB. 7008, from ropy bread	762–764
From Blumberg, Columbia Univ., 1941, Nos. 1, 2, 6, and 7	789–792
From Blumberg, 1941, Nos. 3–5 and 8–10	Discarded
Schweiger, Ark. Univ., 1942, Nos. 1–11, from actinobacillosis-like lesions in cattle and sheep (*141*)	Discarded
From Townsend and Zuch, Calif. Univ. and NCA, 1943, Nos. D, 11, 12, 14, 19, 21, and 23, from incompletely sterilized materials (*166*)	1063, 1073, 1074, 1076, 1081, 1083, and 1085
From Curran, 1944, No. 6, American Can Co.	T1

From Curran, 1944, No. LB	T2
From Bohrer, NCA, 1944, Nos. M43, E109, and M23	T43, T46, and T50
From Lewis, New York Hosp., 1944, strain X	1091
From Dosdall, Minn. Univ., 1944, No. C8j	1110
From Waksman, N. J. Agr. Expt. Sta., 1944, stains PT and ST	1112 and 1113
From Henry, Alberta Univ., Canada, 1946	1143
From Thayer, USPHS, 1946, No. H_7, subtenolin strain (*74*)	1146
From Bohrer, 1947, Nos. M10, M19, M12, M1, and 51E	T112, T116, T122, T125, and T129
From Waksman, 1947; Dostrovsky	1220
From Webley, Aberdeen Univ., Scotland, 1947, strains AFT, EFT, FFT, and HFT (*175*)	T157 and T161–T163
From Kornegay and Youngs, Duke Univ. Med. School, 1947, Nos. D5K, D24, D47, D51, and D53	1226–1230
From Bohrer, 1947, No. C-1889	T193
From Vaichulis, Manteno State Hosp., Ill., 1948, Nos. 19A, 20, 22, 23A, and 26	1243–1247
From Colio, Wyeth Inst., 1949, No. E1, bacillomycin strain (*92*)	1270
From McLeod, USPHS, 1949, Nos. 9–35	1277
From Veg. Lab., USDA, 1949, from incompletely sterilized peas (one of the few cultures that grew fairly well in 12-percent concentration of NaCl)	1279
From Wachter, Purdue Univ., 1950	1310

Two isolations made by the writers from soil and slimy cake were identified as *B. subtilis* (Nos. 242 and 713, respectively).

Bacillus subtilis var. *aterrimus*

Bacillus subtilis var. *aterrimus* Smith, Gordon, and Clark, U. S. Dept. Agr. Misc. Pub. 559: 64. 1946.

Potato bacillus Biel, Centbl. f. Bakt. [etc.] Abt. 2, 2: [137]. 1896.

B. mesentericus niger Lunt, Centbl. f. Bakt. [etc.] Abt. 2, 2: 572. 1896.

B. aterrimus Lehmann and Neumann, Atlas und Grundriss der Bakt., p. 303. 1896.

In 1896 Lehmann and Neumann gave the name *Bacillus aterrimus* to the potato bacillus of Biel and to *B. mesentericus niger* Lunt, both of which had been reported that same year. The identity of *B. aterrimus* was generally confused with that of *B. niger* (*B. lactis niger* Gorini) until the investigation of Clark and Smith (*31*) in 1939. They reported that *B. aterrimus* blackened only carbohydrate mediums, whereas *B. niger* produced black pigment only in the presence of tyrosine.

In the section dealing with Studies on Dissociation it was demonstrated that both of these organisms, under certain conditions, lost their ability to form black pigments (pl. 1, *B*). The colorless dissociants were identical with *B. subtilis* in morphology, physiology, and in the character of growth. They remained colorless once they were stabilized.

The description of *B. subtilis* serves for var. *aterrimus* with the additional statement that a blue-black to black pigment was formed in mediums containing a carbohydrate readily utilized by the organism.

TYPE CULTURES STUDIED

The following type culture was found to conform to the characterization of *Bacillus subtilis* var. *aterrimus.*

Bacillus aterrimus

No. 653, from Cameron, NCA, 1937; Breed; NCTC No. 2591; Ford No. 5B.

This was the only authentic named culture obtained that still exhibited the black pigment.

From Hall, Colo. Univ. Med. School, 1936, No. 798P: Ford. See *B. subtilis* No. 263.
From ATCC, 1936, No. 945; Levine No. 2081. See *B. pumilus* No. 266.
From Schooley, Bucknell Univ., 1936. See *B. megaterium* No. 267.
From NCTC, 1937, No. 2590; Ford No. 5A. See *B. subtilis* No. 330.
From Cameron, NCA, 1937; Breed; NCTC No. 2590; Ford No. 5A. See *B. subtilis* No. 652.
From ATCC, 1939, No. 944; Levine No. 2025. See *B. cereus* No. 722.
From ATCC, 1939, No. 4509; Ford No. 5. See *B. cereus* No. 723.
From Porter, Iowa Univ., 1940; NCTC No. 2590; Ford No. 5A. See *B. subtilis* No. 814.

SYNONYMY

Labeled cultures that apparently corresponded to their original descriptions were identified as *Bacillus subtilis* var. *aterrimus* as follows.

Bacillus nigrificans Fabian and Nienhuis, Mich. Agr. Expt. Sta. Tech. Bul. 140: 23. 1934.

	B. subtilis var. *aterrimus* No.
From Porter, Iowa Univ., 1937; Fabian	624
From Cameron, NCA, 1937; Fabian	659
From ATCC, 1939, No. 4925; Fabian	740

Fabian and Nienhuis reported that *B. nigrificans* was similar to *B. niger* and *B. mesentericus* in many respects and that its distinguishing character was the formation of a water-soluble black pigment in glucose brine. The above three cultures, all originally from Fabian, blackened carbohydrate mediums and were clearly authentic.

From Porter, 1937; Fabian. See *B. pumilus* No. 647.

ASSIGNMENT OF A NAMED CULTURE

The following strain was not recognizable from its description and was identified as *Bacillus subtilis* var. *aterrimus.*

Bacillus tyrosinogenes Carbone and others, Bol. Ist. Sieroterap. (Milan) 2: 29. 1921.

From Hall, Colo. Univ. Med. School, 1937; Ist. Sieroterap., Milan .... *B. subtilis* var. *aterrimus* No. 353

This organism is not to be confused with the anaerobe, *Clostridium tyrosinogenes* (Hall) Bergey and others (*14*). In their study of the serology of *B. aterrimus, B. mesentericus fuscus,* and *B. carotarum,* Carbone and others included *B. tyrosinogenes* and stated that it had been isolated by Rusconi in 1912. The serological results indicated a close relation among all four species, but they provided no other clue as to the identity of *B. tyrosinogenes.* This was the only reference to Rusconi's organism seen by the writers.

IDENTIFICATION OF UNNAMED CULTURES

The following unnamed cultures were identified as *Bacillus subtilis* var. *aterrimus.*

Unnamed cultures	*B. subtilis* var. *aterrimus* No.
From Thom, USDA, 1936, from cornstarch in 1912	230
From Hall, Colo. Univ. Med. School, 1936, Nos. 581, 620, 621A, and 622	259–262

Three strains of *B. subtilis* var. *aterrimus* (Nos. 274–276) were isolated by the writers from soil and air and another strain (No. 748) from decomposed wheat grain.

Bacillus subtilis var. *niger*

Bacillus subtilis var *niger* Smith, Gordon, and Clark, U. S. Dept. Agr. Misc. Pub. 559: 66. 1946.

B. niger Migula, Syst. der Bakt., p. 363. 1900.

B. lactis niger Gorini, Centbl. f. Bakt. [etc.] Originale (1) 20: 94. 1896; R. Soc. Ital. d'Ig. Gior. 16: 9. 1894.

Gorini first named his organism *Bacillus nero,* then changed it to *B. lactis niger.* He described it as a large rod, 1.3μ by 5μ to 7μ, that blackened potato and agar. Later the culture was divided into A and B strains. Two cultures of strain 1A, when examined by the writers, proved to be *B. cereus* (Nos. 256 and 654), fermenting lactose and corresponding to Gorini's original account except for the lack of pigment. Number 1B was smaller (0.7μ by 2μ to 4μ). Two strains of it were studied, one being a typical *B. subtilis* (No. 255) and the other *B. licheniformis* (No. 913).

In 1895 Lehmann and Neumann (*95, p. 617*) failed to get pigment formation by Gorini's culture obtained through Kral's laboratory. On the other hand, Lawrence and Ford (*94*) received from the same source a culture of *B. niger* that blackened nutrient agar. The culture obtained through Hall (No. 264 below) and also Kral's culture from the AMNH (No. 220) still formed the black pigment when examined by the writers. To add to the confusion, Chester (*24, p. 306*) listed Biel's black potato bacillus (*B. aterrimus*) as *B. niger.*

The characterization of *B. subtilis* serves for *B. subtilis* var. *niger* by adding the words, "substrate blackened," to the description of the growth on mediums containing tyrosine.

TYPE CULTURES STUDIED

The following four authentic cultures were identified as *Bacillus subtilis* var. *niger.*

Bacillus niger

No. 220, from AMNH, 1923, No. 733; Kral.
No. 264, from Hall, Colo. Univ. Med. School, 1936, No. 799; Ford; Kral.
No. 650, from Cameron, NCA, 1937; Breed; NCTC No. 2736; Gorini.
No. 651, from Cameron, 1937; Breed; NCTC No. 2592; Ford No. 6.

SYNONYMY

The following three named cultures agreed with their descriptions and were identified as *Bacillus subtilis* var. *niger.*

Bacillus lactis niger	*B. subtilis* var. *niger* No.
From Gorini, Milan, Italy, 1936, No. 2	254
From Cameron, NCA, 1937; Breed; Gorini No. 2	655

Bacillus subtilis (*niger*)

From Rakieten, Hoagland Lab., Brooklyn, N. Y., 1937, No. C3 ....*B. subtilis* var. *niger* No. 704

From Gorini, Milan, Italy, 1936, No. 1B. See *B. subtilis* No. 255.
From Gorini, 1936, No. 1A. See *B. cereus* No. 256.
From Cameron, NCA, 1937; Breed; Gorini No. 1A. See *B. cereus* No. 654.
From Cameron, 1937; Breed; Robertson Nos. 1 and 2. See *B. pumilus* Nos. 657 and 658.
From Porter, Iowa Univ., 1940; Fabian; Gorini. See *B. licheniformis* No. 913.

ASSIGNMENT OF NAMED CULTURES

The following cultures were classified as *Bacillus subtilis* var. *niger.*

Bacillus globigii

	B. subtilis var. *niger* No.
From Bacon Labs., Watertown, Mass., 1947, Red strain; Phillips; McCoy	1221A
From Bacon Labs., 1947, Brown strain; Phillips	1221B

See synonymy of *B. licheniformis.*

IDENTIFICATION OF UNNAMED CULTURES

One unnamed culture (No. 265) from Hall, Colo. Univ. Med. School, 1936, and 21 isolations, Nos. 211–219, 221–229, 253, 356, and 357, made by the writers from soil and air were also identified as *Bacillus subtilis* var. *niger.*

Bacillus pumilus

Bacillus pumilus Gottheil, Centbl. f. Bakt. [etc.] Abt. 2, 7: 681. 1901.

Chester (*25, p. 87*) and Lawrence and Ford (*94, p. 300*) regarded *Bacillus pumilus* as identical with their cultures of *B. mesentericus.* Unfortunately, the strains of *B. mesentericus* that these American investigators had were not the same as the European strains. The European strains, first described by Flügge as *B. mesentericus fuscus* in 1886 (*51*) and named *B. mesentericus* by Trevisan in 1889 (*167*), produced a brown, rugose growth, fermented starch, and reduced nitrates to nitrites. In 1944 Gibson (*63*) noted the similarity between the European *B. mesentericus* and *B. subtilis* and recommended that they be listed in synonymy.

In agreement with Chester (*25*) and Lawrence and Ford (*94*), the American strains of *B. mesentericus* were found (*147*) to be the same as *B. pumilus,* a species reported by Gottheil and recognizable from his account. Because of this confusion between the American and European strains of *B. mesentericus* it was suggested (*147*) that the name *B. mesentericus* be considered *nomen dubium* and that the American strain of *B. mesentericus* be known hereafter as *B. pumilus.*

Gibson (*63*) reluctantly recognized *B. pumilus* as a species and separated it from *B. subtilis* by starch hydrolysis and nitrate reduction. The latter test was not entirely reliable in his hands. Knight and Proom (*84*), however, reported that *B. pumilus* and *B. subtilis* differed in that the former required biotin for growth in an ammonia basal medium. Information on the egg-yolk (lecithinase) reaction, on the other hand, failed to give a clear separation, Colmer (*34*) and McGaughey and Chu (*110*) having recorded a negative test and Knight and Proom a restricted reaction, which they considered might or might not have been due to lecithinase. It must be readily admitted that *B. subtilis, B. licheniformis,* and *B. pumilus* are very closely related and still need additional study.

The following description of *B. pumilus* was based on the examination of 4 type cultures, 6 strains that bore names in synonymy, 45 cultures not considered authentic, and 38 unnamed cultures, making a total of 93 strains.

CHARACTERS

**Vegetative rods.*—0.6μ to 0.7μ by 2.0μ to 3.0μ; evenly stained; ends rounded; motile; no chains, capsules, or shadow-forms. *Variations:* Chains and filaments; encapsulated; shadow-forms; or Gram-variable.

On glucose-nutrient agar the cells contained a few small fat globules. There was a tendency toward larger cells, more filaments, and Gram-negative forms.

**Sporangia.*—Not definitely bulged.

**Spores.*—0.5μ by 1.0μ; oval to cylindrical; central or paracentral; thin-walled. *Variations:* 0.6μ to 0.8μ by 0.8μ to 1.5μ; or sporulation slow. (Two strains sporulated only on soybean agar.)

Colonies.—Smooth; thin; flat; spreading; dendroid; translucent. *Variations:* Small; nonspreading; dense, or pinpoints.

Nutrient agar slants.—Growth smooth; thin; glistening; spreading; nonadherent; frequently yellowish. *Variations:* Rough; dull; tough; or wrinkled.

Nutrient broth.—Turbidity uniform, with or without a ring or fragile pellicle. *Variations:* Turbidity flocculent; or broth clear with rough pellicle.

**NaCl broth.*—Good growth in 5-percent concentration of NaCl; usually growth in 7-percent; growth in a few cases in 10-percent.

**Glucose-nutrient agar slants.*—Growth usually similar to that on nutrient agar. *Variations:* Heavier; or scantier.

**Tyrosine agar slants.*—Growth same as on nutrient agar.

**Utilization of citrate.*—Positive.

Potato.—Growth smooth; thin; soft; spreading; moist to slimy; yellow to brown; potato darkened. *Variations:* Growth rough; dry; wrinkled; pink; or red.

**Soybean agar slants.*—Growth more abundant than on nutrient agar; soft; yellow. *Variations:* Growth rough; red; or colorless.

Glucose-nitrate agar slants.—Growth usually scant. *Variations:* Good; or no growth.

**Glucose-asparagine agar slants.*—Growth abundant. *Variations:* Scant; or no growth.

**Hydrolysis of starch.*—Negative.

**Production of acetylmethylcarbinol.*—Positive (incubation temperature 32° C.). *Variation:* One strain (No. 736) negative.

pH of glucose broth.—Variable, 5.0 to 8.4.

Fermentation tests.—Acid without gas from arabinose, xylose, glucose, sucrose, and mannitol; usually negative on lactose.

**Hydrolysis of gelatin.*—Positive; wide zone of hydrolysis.

Hydrolysis of casein.—Positive; usually wide zone of clearing.

**Reduction of nitrate to nitrite.*—Negative.

**Anaerobic production of gas from nitrate.*—Negative.

**Anaerobic growth in glucose broth.*—Very scant, if any, growth; pH 5.5 or higher at 14 days.

Temperatures for growth.—Good growth at 28° to 40° C.; maximum temperature of growth 45° to 50°.

TYPE CULTURES STUDIED

The following authentic cultures were received and identified as *Bacillus pumilus.*

Bacillus pumilus

No. 272, from Löhnis, Leipzig, 1914; Kral.
No. 630, from Porter, Iowa Univ., 1937; Bredemann.
No. 939, from Porter, 1940; Bredemann, strain a.
No. 940, from Porter, 1940; Stapp.

From ATCC, 1939, No. 4344. See *B. subtilis* No. 741.
From Porter, 1940; Claussen. See *B. subtilis* No. 941.
From Porter, 1940; Claussen. See *B. cereus* No. 942.

SYNONYMY

The following named strains were identified by the writers as *Bacillus pumilus.*

Bacillus mesentericus, American strain, Chester, Del. Agr. Expt. Sta. Ann. Rpt. (1903): 42. 1904.

	B. pumilus No.
From AMNH, 1923, No. 726; Ford	236
From Soule, Mich. Univ., 1936	307
From ATCC, 1939, No. 945; Levine No. 2081	738
From Porter, Iowa Univ., 1940; Edwards; ATCC	896

Bacillus mesentericus flavus Laubach, Jour. Bact. 1: 497. 1916.

	B. pumilus No.
From NCTC, 1937, No. 2595; Ford No. 9	333
From ATCC, 1939, No. 4520; Ford No. 9	739

Laubach named this variety of the American strain of *B. mesentericus* because of its yellow pigment. The writers, however, found this character not unusual among cultures of the species.

ASSIGNMENT OF OTHER NAMED CULTURES

The 45 cultures identified below as *Bacillus pumilus* did not conform to or were not recognizable from their original descriptions.

Bacillus adhaerens Laubach, Jour. Bact. 1: 503. 1916.

	B. pumilus No.
From ATCC, 1936, No. 1; AMNH; Ford	309
From NCTC, 1937, No. 2604; Ford No. 18	337

Laubach stated that the colonies of this organism somewhat resembled those of *B. mycoides;* growth on agar slants extended into the medium; and the diameter of the spores was greater than that of the rods. These characters did not apply to the cultures at hand.

From Porter, Iowa Univ., 1940; Bredemann. See *B. subtilis* No. 802.

Bacillus agri Laubach and Rice, Jour. Bact. 1: 516. 1916.

From NCTC, 1937, No. 2598; Ford No. 12 .... *B. pumilus* No. 336

The original account of this species is incomplete and does not apply in all respects to any of the strains studied.

From ATCC, 1936, No. 4507; Ford No. 12. See *B. cereus* No. 310.
From ATCC, 1936, No. 2; AMNH No. 800; Ford. See *B. cereus* No. 342.
From Lamanna, Camp Detrick, 1947, No. 13. See *B. brevis* No. 1219.

Bacillus agrophilus Stührk, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 189. 1935.

From Porter, 1940; Bredemann .... *B. pumilus* No. 807

Stührk's description of this organism, although incomplete, seems to indicate that it was similar to *B. brevis*.

Bacillus aterrimus

From ATCC, 1936, No. 945; Levine No. 2081 .... *B. pumilus* No. 266

For authentic cultures, see *B. subtilis* var. *aterrimus*.

Bacillus brevis

From ATCC:	*B. pumilus* No.
1939, No. 18; AMNH; Ford	724
1939, No. 4510; Ford No. 27	725

See *B. brevis* below in group 2.

Bacillus carotarum

From Porter, 1940; Stapp .... *B. pumilus* No. 827

See *B. carotarum* in synonymy of *B. megaterium*.

Bacillus cohaerens

From NCTC, 1937, No. 2596; Ford No. 10A; Kral.... *B. pumilus* No. 334

For a discussion of this species see assigned cultures under *B. megaterium*.

Bacillus dendroides Holzmüller, Centbl. f. Bakt. [etc.] Abt. 2, 23: 304. 1909.

From Porter, 1940; NCTC No. 927; Thornton .... *B. pumilus* No. 844

Thornton (*162*) believed that his isolation was similar to *B. dendroides* and that it belonged to the *B. subtilis* group. He also stated that his strain hydrolyzed starch and reduced nitrates to nitrites. Culture No. 844, a typical *B. pumilus*, is not, therefore, Thornton's original strain.

Bacillus fastidiosus Dooren de Jong, Zentbl. f. Bakt. [etc.] Abt. 2, 79: 350. 1929.

From Porter, 1937; Dooren de Jong No. 1071........ *B. pumilus* No. 643

This culture bore no resemblance to the original organism, a large (2.0μ in diameter) Gram-negative rod that would not grow on peptone and utilized only the carbon from uric acid.

Bacillus fusiformis

	B. pumilus No.
From AMNH, 1920, No. 732	247
From ATCC, 1939, No. 61; Ford No. 10	727A
From Porter, 1940; Bredemann	864
From Porter, 1940; Stapp	865

In 1937 culture No. 247 was separated from *B. fusiformis* No. 350 (see synonymy of *B. sphaericus*) and in 1947 No. 727A was isolated from No. 727 (see *B. circulans*). For authentic cultures of *B. fusiformis*, see *B. sphaericus* in group 3.

Bacillus globigii

From NCTC, 1937, No. 2593; Ford No. 7; Kral ..... *B. pumilus* No. 331

See synonymy of *B. licheniformis.*

Bacillus lacticola

From Porter, 1940; Claussen .... *B. pumilus* No. 878

See *B. lacticola* in synonymy of *B. cereus.*

Bacillus liodermis Flügge, Ztschr. f. Hyg. u. Infektionskrank. 17: 296. 1894; Die Mikroorganismen, p. 323. 1886.

From ATCC, 1939, No. 70; AMNH; Winslow .... *B. pumilus* No. 734

Flügge's account is too meager for the identification of his culture. Chester (*24, p. 272*) and Lehmann, Neumann, and Breed (*96, p. 619*) considered this species closely related to *B. vulgatus* (*B. subtilis*).

Bacillus luteus

From Porter, 1940; Stapp .... *B. pumilus* No. 885

See assignment of other named cultures under *B. cereus.*

Bacillus malabarensis

From Porter:	*B. pumilus* No.
1940; Bredemann	620
1940; Claussen	891

See *B. malabarensis* in synonymy of *B. megaterium.*

Bacillus megaterium

From ATCC:	*B. pumilus* No.
1939, No. 71; AMNH No. 734A; Ford	735
1939, No. 72; Ford; Kral	736

For authentic cultures see *B. megaterium*, the first species described above. Culture No. 736 was the only strain of *B. pumilus* in this collection that did not produce acetylmethylcarbinol.

Bacillus niger

From Cameron, NCA, 1937; Breed; Robertson Nos. 1 and 2 .... *B. pumilus* Nos. 657 and 658

Authentic cultures are listed under *B. subtilis* var. *niger.*

Bacillus nigrificans

From Porter, 1937; Fabian .... *B. pumilus* No. 647

The above strain of *B. pumilus* occurred as a contaminant of the original culture. See *B. subtilis* var. *aterrimus* No. 624.

Bacillus panis

	B. pumilus No.
From Blumberg, Columbia Univ., 1941	Discarded
From ATCC, 1936, No. 4522; Ford No. 8	316

A discussion of *B. panis* will be found in the synonymy of *B. subtilis.*

Bacillus parvus

From Porter:	*B. pumilus* No.
1937; Bredemann	629
1940; Claussen	928

For an authentic culture, see synonymy of *B. subtilis.*

Bacillus pseudotetanicus

From Porter, 1940; NCTC No. 2609; Ford No. 25 .... *B. pumilus* No. 937

See discussion of *B. pseudotetanicus* in the introduction to *B. sphaericus* in group 3.

Bacillus ruminatus

From ATCC, 1936, No. 98; AMNH No. 793; Ford ... *B. pumilus* No. 345

See synonymy of *B. megaterium.*

Bacillus sublustris Schieblich, Centbl. f. Bakt. [etc.] Abt. 2, 58: 206. 1923.

From Porter, 1940; Schieblich .... *B. pumilus* No. 980

According to Schieblich, the vegetative cells of his organism were 0.7μ to 1.0μ in diameter, had rounded ends, and usually swelled before the round terminal spores appeared. The growth was restricted, smooth, and shiny on agar; no acid or gas was formed from glucose. All these characteristics point to *B. sphaericus*, certainly not to *B. pumilus.*

Bacillus subtilis

From Delaporte, Lab. Cytol. Vég., Paris, 1949, Lister, hay, and Marburg strains .... *B. pumilus* Nos. 1299–1301

For authentic cultures, see *B. subtilis* above.

Bacillus teres

From Porter, 1937; Bredemann .... *B. pumilus* No. 637

See discussion under assigned cultures of *B. subtilis.*

Bacillus terminalis var. *thermophilus* Prickett, N. Y. [Geneva] Agr. Expt. Sta. Tech. Bul. 147: 44. 1928.

From Porter, 1940; NCTC No. 2688; Conn .... *B. pumilus* No. 984

Prickett stated that this variety had all the characters of *B. terminalis* Migula (*115, p. 578*) except for a brown coloration on agar and a much higher optimum temperature for growth (55° to 65° C.). On the other hand, culture No. 984 was inhibited at 45°, did not grow at 50°, and produced a yellow pigment on potato.

Bacillus terrestris Werner, Zentbl. f. Bakt. [etc.] Abt. 2, 87: 461. 1933.

From Porter, 1937; Bredemann .... *B. pumilus* No. 638

According to Werner the spores of his organism were round and the shape of the sporangium was drumstick. Number 638 is, therefore, not authentic.

Bacillus thermophilus Rabinowitsch, Ztschr. f. Hyg. u. Infektionskrank. 20: 154. 1895.

	B. pumilus No.
From Porter, 1940; NCTC No. 2812; Pribram; Nègre	982
From McCoy, Wis. Univ., 1945	T84

Nègre (*118*) identified his isolate from sand of the Sahara as *B. thermophilus* and described it as having an optimum temperature for growth of 50° C. and a maximum of 70°. Numbers 982 and T84 had an optimum temperature for growth of 28° to 40° and failed to grow at 52°.

Bacillus tritus Batchelor, Jour. Bact. 4: 29. 1919.

From Henry, Wash. (State) Univ., 1937, No. 193 ... *B. pumilus* No. 667

Batchelor wrote that this species did not liquefy gelatin or form acid from glucose or sucrose. Except for these properties, culture No. 667 was not at variance with her rather brief characterization.

Bacillus truffauti Truffaut and Bezssonoff, Acad. des Sci. Colon. [Paris], Compt. Rend. 175: 544. 1922.

	B. pumilus No.
From McCoy, 1937; Truffaut	355
From Porter, 1940; NCTC No. 1490	997

The organism of Truffaut and Bezssonoff reduced nitrates to nitrites, hydrolyzed starch, formed a pellicle on broth, and was said to fix atmospheric nitrogen. Aside from the fixation of nitrogen, these and other characters given point to *B. subtilis* rather than *B. pumilus.*

Bacillus tumescens

From Porter, 1940; NCTC No. 2607; Ford No. 5A . . *B. pumilus* No. 990

See synonymy of *B. megaterium.*

IDENTIFICATION OF UNNAMED CULTURES

The following 38 unnamed cultures were submitted to the writers during the course of the work and have been identified after a complete study as strains of *Bacillus pumilus.*

Unnamed cultures	*B. pumilus* No.
From NIH, 1937, Nos. 5, 43, 44, 56, 197, 201, and 212	576–578, 580, and 582–584
From Burkey, USDA, 1938, Nos. 1, 2, 4, 5, 12, and 14	706–711
From Branch, McGill Univ., 1940, Nos. 2, 18, 595, and 801	Discarded
From Ark, Calif. Univ., 1941	788
From Weintraub, Smithsonian Inst., 1943	1034 and 1035
From Townsend and Zuch, Calif. Univ., and NCA, 1943, Nos. 1–5 (*166*)	1065–1069
From Bohrer, NCA, 1944, No. EB20	T45
From Reuszer, USDA, 1946	1166
From Bohrer, 1947, Nos. 1176 and C-1479	T113 and T2003
From Delaporte, Lab. Cytol. Vég., Paris, 1949, Nos. 8B3, 9J1, 11B17, 13R1, 731, 6C3, and 11	1293–1298 and 1302
From Hansen, Md. Univ., 1950, No. 5239-b	1311
From O'Brien, USDA, 1950	1336

Bacillus coagulans

Bacillus coagulans Hammer, Iowa Agr. Expt. Sta. Res. Bul. 19: 129. 1915; Sarles and Hammer, Jour. Bact. 23: 301. 1932.

Bacillus coagulans was introduced by Hammer in 1915 as the cause of an outbreak of coagulation in evaporated milk. In 1932 Sarles and Hammer made a more detailed study of the organism and reported that an unusual quantity of acid was formed from carbohydrates, the nonvolatile fraction being d-lactic acid and the volatile portion apparently acetic and propionic acids.

The 16 strains of *B. coagulans* examined by Knight and Proom (*84*) required amino acids, biotin, and aneurin (thiamine) for growth. They could not substantiate the observation that nicotinic acid was essential, as given by Cleverdon and others (*32*). The egg-yolk (lecithinase) reaction of Knight and Proom's strains was negative, corroborating McGaughey and Chu's report (*110*) on 4 cultures.

The strains previously studied by Gordon and Smith (*65*) seemed to be intermediate between the mesophilic and thermophilic sporeformers. They preferred a temperature of 33° to 45° C., growing poorly, if at all, at 28° and some of them growing at 60°. (See section on Temperature Relationships of Species.) In their microscopic appearance they resembled *B. subtilis,* although the rods were usually longer, the spores subterminal to terminal, and the sporangia in some cases definitely swollen. This morphology, therefore, placed *B. coagulans* intermediate between groups 1 and 2 (fig. 2) and made its allocation in a key primarily based on morphological features extremely difficult. Its assignment to group 1 in the key in close proximity to *B. subtilis* was perhaps arbitrary, and future study may reveal a more suitable location. Fortunately, the physiology of *B. coagulans* proved to be distinctive and furnished a comparatively reliable means for the recognition of the species.

The following description of *B. coagulans* was based on 6 type cultures, 17 cultures that bore names in synonymy, 5 named cultures that did not conform to their original descriptions, 22 that were received unnamed, and 27 isolated by the writers, making a total of 77. Unless otherwise noted, 37° C. was used for the incubation of all cultures.

CHARACTERS

**Vegetative rods.*—0.6μ to 1.0μ by 2.5μ to 5.0μ; not in chains; uniformly stained; motile; Gram-positive. *Variations:* 0.5μ to 1.2μ by 2.0μ to 6.0μ; filaments; or Gram-variable.

**Sporangia.*—Definitely swollen in some cases; not swollen in others.

**Spores.*—0.9μ to 1.0μ by 1.2μ to 1.5μ; oval; thin-walled; subterminal to terminal. *Variations:* 0.8μ to 1.1μ by 1.2μ to 2.0μ; bent (No. 796); or cylindrical. (As a rule spores were formed less abundantly than by other members of group 1. Attempts to increase sporulation by varying the nutrients were not successful. See also Schmidt (*140*).)

Colonies.—Usually small; round; opaque; not distinctive.

Nutrient agar slants.—Growth variable; scant to moderate; flat; smooth to rough; translucent to opaque.

**NaCl broth.*—No growth in 5-percent concentration of NaCl.

**Glucose-nutrient agar slants.*—Growth soft; moist; more abundant than on nutrient agar. *Variation:* No stimulation of growth by glucose in the case of seven strains.

**Utilization of citrate.*—Negative.

Potato.—Growth erratic.

**Soybean agar slants.*—Growth usually heavier than on nutrient agar. *Variation:* Growth of 14 strains same as on nutrient agar.

**Proteose-peptone acid agar slants.*—Good growth. (According to Stern, Hegarty, and Williams (*158*), this medium supported better sporulation than nutrient agar.)

**Stock-culture agar slants.*—Growth as good as or better than on nutrient agar. *Variation:* Growth scantier (Nos. T139, T140, T142, and T2006).

**Glucose-asparagine agar slants.*—Growth scant, if any. *Variation:* Good growth (No. 905).

Tomato-yeast milk.—Curdled in 1 to 3 days at 45° C. *Variation:* No curd (Nos. T200 and T2006).

**Hydrolysis of starch.*—Positive. *Variation:* One strain (No. T139) negative.

Production of acetylmethylcarbinol.—Usually positive (incubation temperature 37° C.). *Variation:* Approximately 27 percent of strains negative.

pH of glucose broth.—5.0 or less at 7 days.

Fermentation tests.—Acid without gas from glucose; acid variable from arabinose, xylose, lactose, sucrose, glycerol, and mannitol. (Organic nitrogen was preferred by all strains of this species.)

**Hydrolysis of gelatin.*—Negative or weak. *Variation:* Good hydrolysis by one strain (No. T115).

Hydrolysis of casein.—Negative or weak.

Reduction of nitrate to nitrite.—Usually negative. *Variation:* Eighteen strains positive.

Anaerobic production of gas from nitrate.—Negative.

Anaerobic growth in glucose broth.—Positive; pH below 5.2. (A few strains were inhibited by the alkalinity of the broth generally used. In the case of this species a neutral broth, therefore, is recommended.)

**Temperatures for growth.*—Good growth from 33° to 45° C.; maximum temperature for majority of strains 55° to 60°; no growth at 65°; poor growth, if any, at 28°.

TYPE CULTURES STUDIED

The following authentic cultures were received and examined.

Bacillus coagulans

No. 609, from Porter, Iowa Univ., 1937; Hammer.
Nos. 795–798, from Hammer, Iowa State Col., 1941, Nos. 195, 196, 198, and 200.
No. T13, from Curran, USDA, 1944, No. 195; Iowa State Col.
From Porter, 1940; Iowa State Col. See *B. cereus* No. 833.

SYNONYMY

The following named cultures that conformed to their descriptions were identified as *Bacillus coagulans.*

Bacillus dextrolacticus Andersen and Werkman, Iowa State Col. Jour. Sci. 14: 187. 1940.

From Werkman, Iowa State Col., 1941 .... *B. coagulans* No. 784

In describing their new species, Andersen and Werkman noted that their organism was related to *B. coagulans* but differed from it in reducing nitrates to nitrites and forming acid from arabinose and sorbitol. They stated also that it formed more acetylmethylcarbinol and more d-lactic acid from glucose. Although very active originally, culture No. 784, when studied by the writers, failed to utilize sorbitol and did not differ markedly in any respect from other strains of *B. coagulans.*

Bacillus thermoacidificans Renco, Ann. Microbiol. 2: 109. 1942.

From Renco, Ist. Sperimentale di Caseificio di Lodi, Milan, 1947	*B. coagulans* No. T186
From Olsen, Danmarks Tekniske Højskole, Copenhagen, 1948	*B. coagulans* No. T2011

Immediately after Renco's publication on this species, Gorini (*67*) stated that he believed *B. thermoacidificans*, as well as *B. calidolactis* Hussong and Hammer (*76*), were the same as his *B. lactis termophilus* (*66*). He failed, however, to mention what the relationship of these species might be to *B. coagulans*. As the name of Gorini's species is a trinomial and the identity of *B. calidolactis* open to question (see below), the writers cannot support Gorini's claim of priority.

Renco's culture obtained directly from him and also through Olsen proved to be identical with *B. coagulans*.

Bacillus thermoacidurans Berry, Jour. Bact. 25: 72. 1933.

	B. coagulans No.
From Hegarty, NCA, 1940	770
From Pilcher, Amer. Can Co., 1944, Nos. 94S, 81S, 8T, 4E, 6, 43P, and 78G	T21–T27
From Brooks, N. Y. [Geneva] Agr. Expt. Sta., 1947; Amer. Can Co., No. 20	T2005
From NCTC, 1948, No. 3991; Berry	T2021
From Bohrer, NCA, 1949; Gibson, vars. A and B	T2022 and T2023

Berry named and fully described this organism isolated from off-flavor tomato juice, and, as a result, *B. thermoacidurans* became well known in the canning industry before its identity with *B. coagulans* was recognized (*147*). In confirmation, Becker and Pederson (*9*), after a careful study of the two species, stated, "There is no justification for considering *B. thermoacidurans* as a species distinct from *B. coagulans* and the latter name has priority. The various morphological and physiological characteristics, as well as the end products of growth, are all identical or within the degree of variability of different strains" of the species.

Lactobacillus cereale Olsen, Kemisk 25: 125. 1944.

From Olsen, 1948 strains U and B	*B. coagulans* Nos. T2012 and T2013

Olsen observed several minor differences between these two sporeformers isolated from fermenting barley. When studied in the writers' laboratory, however, they could not be distinguished from each other or from other strains of *B. coagulans*.

ASSIGNMENT OF NAMED CULTURES

The following cultures did not conform to or were not recognizable from their original descriptions and were identified as *Bacillus coagulans*.

Bacillus calidolactis Hussong and Hammer, Jour. Bact. 15: 179. 1928.

From Brooks, N. Y. [Geneva] Agr. Expt. Sta., 1947; Iowa State Col., strains C1, O1, and sp	*B. coagulans* Nos. T2006–T2008

Of the five cultures of *B. calidolactis* that originally came from Iowa State College, three were identified as *B. coagulans* and two as *B. stearothermophilus*. Unfortunately, the temperatures for growth given in the original description are clearly those of *B. stearothermophilus*, whereas several of the remaining characters are those of *B. coagulans*.

From Porter, Iowa Univ., 1940; NCTC No. 2690. See *B. licheniformis* No. 821.
From Curran, USDA, 1944, unnumbered and No. 11; Iowa State Col. See *B. stearothermophilus* Nos. T15 and T16.

Bacillus modestus Schieblich, Zentbl. f. Bakt. [etc.], Originale (1), 124: 273. 1932.

From Porter, 1940; Schieblich	*B. coagulans* No. 905

According to the original account, this organism was 1.0μ to 1.2μ in diameter; its protoplasm was granular in appearance; gelatin was liquefied; and alkali produced in milk. These characteristics, as well as the illustration given by Schieblich, indicate that his culture was probably *B. cereus*, certainly not *B. coagulans*.

Lactobacillus delbrueckii (Leichmann) Biejerinck, Arch. Néerland. des Sci. Exact. et Nat., Haárlem, Ser. 2, 7: 212, 1901.

From Pederson, N. Y. [Geneva] Agr. Expt. Sta. 1948, No. 1260; Staley Mfg. Co. .... *B. coagulans* No. T2020

The high acidity normally formed from carbohydrates by *B. coagulans* may be responsible for its confusion with members of the genus *Lactobacillus*.

IDENTIFICATION OF UNNAMED CULTURES

The following 22 unnamed cultures were identified as *Bacillus coagulans.*

Unnamed cultures	*B. coagulans* No.
From Curran, USDA, 1944, No. 1460; NCA	T14
From Bohrer, NCA, 1944, Nos. 4167, 1264, 52–240, 4578, and 4110	T54–T58
From McCoy, Wis. Univ., 1945; Pan, strain E (*124*)	T83
From Bohrer, 1947, Nos. 1215, 1264, 1460, 1734, and 848	T114, T115, T121, T126, and T134
From Bohrer, 1947, Nos. 518, 698, 880, 1150, 11878, 2, and 4	T138–T140, T142–T145
From Bohrer, 1947, Nos. C–1655 and C–1657	T198 and T200
From Pederson, N. Y. [Geneva] Agr. Expt. Sta., 1948	T2016

Twenty-seven cultures were isolated by the writers from cream (Nos. T96–T105), from cheese (Nos. T169–T176 and T190–T192), and silage (Nos. T177, T178, and T181–T184).

Bacillus firmus

Bacillus firmus Werner, Zentbl. f. Bakt. [etc.] Abt. 2, 87: 470. 1933.

Bacillus firmus was difficult to classify because it was morphologically quite similar to *B. pumilus* or *B. subtilis* but physiologically quite different. Growth was slower, less abundant, and greatly inhibited by glucose; nitrogen requirements were more complex; and acid tolerance was low. As a result, a great many of the physiological characters of this species were found to be negative. The 19 strains available for study presented a rather uniform pattern that may or may not prove to be representative of the species when more strains are examined. The 5 cultures of *B. firmus* investigated by Knight and Proom (*84*) had a complex nutrition pattern and a negative egg-yolk (lecithinase) reaction.

Eight cultures labeled *B. firmus* were obtained through Porter from Bredemann, under whom Werner did his work. Five of them conformed to the description given below for *B. firmus;* the other three were strains of *B. brevis.* The original account is indefinite and may apply to either species. It is assumed, however, that the 5 strains are authentic and that some mistake was made in the case of the 3 *B. brevis* cultures.

The following description of *B. firmus* was based on the study of 5 type cultures, 1 strain with a name in synonymy, 1 culture bearing another name, 11 cultures received without names, and 1 isolation, a total of 19 strains.

CHARACTERS

**Vegetative rods.*—0.6µ to 0.9µ by 1.2µ to 4.0µ; not in chains; ends rounded to slightly pointed; uniformly stained; motile; Gram-positive.

**Sporangia.*—Only slightly swollen, if at all.

**Spores.*—Variable in size; 0.7µ to 1.0µ by 1.0µ to 1.4µ; oval; thin-walled; central to subterminal.

Colonies.—Small; smooth; round; whitish; not distinctive. *Variation:* Pink (Nos. 749, 1152, and 1329).

Nutrient agar slants.—Growth moderate; flat; opaque; ribbonlike; whitish. *Variations:* Spreading; abundant; or pink (Nos. 749, 1152, and 1329).

Nutrient broth.—Turbidity light; uniform; or flocculent.

NaCl broth.—Usually growth in 5-percent concentration of NaCl; no growth in 10-percent.

**Glucose-nutrient agar slants.*—Growth inhibited; very scant compared with that on nutrient agar.

**Utilization of citrate.*—Negative.

**Potato.*—No growth.

**Soybean agar slants.*—Scant, if any, growth.

Proteose-peptone acid agar slants.—No growth.

**Glucose-nitrate agar slants.*—No growth.

**Hydrolysis of starch.*—Positive.

**Production of acetylmethylcarbinol.*—Negative (incubation temperature 32° C.).

**pH of glucose broth.*—Very little, if any, growth; pH at 7 days not less than 6.2.

**Utilization of inorganic nitrogen.*—Very slight, if any.

Fermentation tests.—Small amount of acid without gas from glucose with organic nitrogen.

**Hydrolysis of gelatin.*—Positive.

**Hydrolysis of casein.*—Positive.

Production of indole.—Negative.

**Urease.*—Negative.

Reduction of nitrate to nitrite.—Positive. *Variation:* Two strains negative (Nos. 749 and 1119).

Anaerobic production of gas from nitrate.—Negative.

Anaerobic growth in glucose broth.—Negative.

Reduction of methylene blue.—Negative at 7 days.

Temperatures for growth.—Good growth at 28° to 33° C.; growth at 45° by only one-half of strains; no growth at 50°.

TYPE CULTURES STUDIED

The following type cultures of *Bacillus firmus* were received by the writers.

Bacillus firmus

Nos. 613, 854, 855, 858, and 860, from Porter, Iowa Univ., 1937; Bredemann, strains original, A (Hamburg), B, E (Pyramiden), and G (Dolomiten).

From Porter, 1937; Bredemann, strains C (Rauschen), D (Utilberg), and F (Predigtstuhl). See *B. brevis* Nos. 856, 857, and 859.

SYNONYMY

The following culture agreed with its original description and was found to be identical with *Bacillus firmus.*

Bacillus imomarinus ZoBell and Upham, Scripps Inst. Oceanography Bul. 5: 265. 1944.

From ZoBell, Scripps Inst., 1945, No. 562............*B. firmus* No. 1119

Number 1119 was one of the 2 strains failing to reduce nitrates to nitrites. Although described originally as having an optimum temperature for growth of 20° to 25° C., this culture grew well at 28° to 37°.

ASSIGNMENT OF A NAMED CULTURE

The following culture differed somewhat from its original description and was identified as *Bacillus firmus.*

Bacillus flavidus Stührk, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 185. 1935.

From Porter, Iowa Univ., 1940; Bredemann..........*B. firmus* No. 861

Stührk stated that his organism had an optimum temperature for growth of 38° to 40° C. and did not hydrolyze starch, reduce nitrates to nitrites, or liquefy gelatin. Culture No. 861, however, was positive in all three characters and its optimum temperature was about 30°. It agreed, on the other hand, with Stührk's account as to the size of the rods and spores and the lack of growth on potato.

IDENTIFICATION OF UNNAMED CULTURES

The following 11 unnamed cultures were identified as *Bacillus firmus.*

Unnamed cultures	*B. firmus* No.
From Broadhurst, Columbia Univ., 1939 (one of the 2 strains failing to reduce nitrates to nitrites)	749
From Raper, USDA, 1940, from myxomycete culture	783
From Townsend and Zuch (*166*), Calif. Univ. and NCA, 1943, No. 8	1070
From Fuller, Iowa State Col., 1945	1131 and 1132
From Reuszer, USDA, 1946	1147–1149, 1151, and 1152
From Allen, Hopkins Marine Sta., 1950	1329

In addition, 1 strain of *B. firmus* (No. 769) was isolated by the writers from partially decomposed wheat grain.

Bacillus lentus

Bacillus lentus Gibson, Zentbl. f. Bakt. [etc.] Abt. 2, 92: 368. 1935.

Gibson studied nine strains of *Bacillus lentus* isolated from five different soils and concluded, "They appear to be sufficiently well defined to justify their recognition as a distinct species." He recognized their relation to *B. pasteurii* and the difficulty of classifying these and similar organisms that are negative in so many of their physiological reactions. The two strains (Gibson's Nos. 165 and 238) available to the writers for study seemed to be distinguishable from *B. firmus.* Knight and Proom (*84*), however, observed that their three strains of *B. lentus* resembled cultures of *B. firmus,* in having complex nutritional requirements but differed in their restricted egg-yolk reaction. Pending further examination of more strains, *B. lentus* has been listed as a separate species.

CHARACTERS

The characters listed for *B. firmus* applied to *B. lentus* with the following exceptions:

**Hydrolysis of gelatin.*—Negative.
**Hydrolysis of casein.*—Negative.
**Urease.*—Positive.
Reduction of nitrate to nitrite.—Negative.
Temperatures for growth.—Growth at 28° to 33° C.; no growth at 45°.

TYPE CULTURES STUDIED

The following type cultures were received.

Bacillus lentus

No. 670, from Gibson, Edinburgh Univ., Scotland, 1937, No. 165.
No. 883, from Porter, Iowa Univ., 1940; Gibson No. 165.
No. 1262, from Gibson, 1948, No. 238.

GROUP 2. SPORANGIA DEFINITELY SWOLLEN BY OVAL SPORES

Bacillus polymyxa

Bacillus polymyxa (Prazmowski) Migula, Syst. der Bakt., p. 638. 1900.
Clostridium polymyxa Prazmowski, Inaug. Diss., Leipzig, p. 37. 1880.

Porter, McCleskey, and Levine (*126*) reviewed the literature and made a detailed study of a large number of cultures of aerobic sporeformers that produced gas from carbohydrates. They reported that their cultures belonged to two species—*Bacillus polymyxa* and *B. macerans.* The writers confirmed these findings in all respects, except in the failure of *B. polymyxa* to ferment rhamnose and sorbitol. Most of the strains of this species in the writers' collection produced acid and gas from both substances, and some of them reacted as vigorously as *B. macerans.* Although this was a minor point, an attempt was made to find the reason for this discrepancy in the case of rhamnose. The brand of pure rhamnose recommended by Tilden and Hudson (*163*) was procured and the results were the same as before. It was concluded, therefore, that the fermentation of rhamnose and sorbitol was not a reliable character for the separation of the two species.

Katznelson and Lochhead (*81*), examining 82 strains, and Knight and Proom (*84*), 13 strains, found that *B. polymyxa* required only biotin for growth in an ammonia basal medium. The egg-yolk (lecithinase) reaction according to Colmer (*34*) and McGaughey and Chu (*110*) was negative (a total of 4 strains), whereas Knight and Proom (*84*), testing 13 strains, reported a variable restricted reaction.

The following description of *B. polymyxa* was based on the study of 25 strains, of which 8 were type cultures, 5 bore names in synonymy, 7 were received unnamed, and 5 were isolations from soil.

CHARACTERS

**Vegetative rods.*—0.6μ to 0.8μ by 2.0μ to 7.0μ; not in chains; motile; Gram-variable. *Variation:* 0.9μ to 1.0μ in diameter.

On glucose-nutrient agar the rods were usually larger and contained several small fat globules.

**Sporangia.*—Definitely bulged; spindle-shaped or clavate.

**Spores.*—1.2μ to 1.5μ by 1.5μ to 2.5μ; oval; central to terminal; spore wall usually thick and stainable.

Colonies.—Thin; translucent; spreading; lobate or fimbriate; the rough stage small; round; whitish; sometimes adherent.

Nutrient agar slants.—Growth scant to moderate; restricted or spreading; indistinct to whitish.

Nutrient broth.—Turbidity uniform to granular; gummy sediment.

NaCl broth.—No growth in 5-percent concentration of NaCl. (Knight and Proom (*84*) reported growth in a 4-percent concentration.)

Glucose-nutrient agar slants.—Growth usually much thicker than on nutrient agar; raised; glistening; gummy; with formation of gas. *Variation:* Growth thin and not gummy.

Utilization of citrate.—Negative. *Variation:* Two strains positive.

**Potato.*—Growth moderate to abundant; slimy; whitish to light tan; potato decomposed with formation of gas; growth of rough strains thicker and heaped up.

Soybean agar slants.—Good growth; usually formation of gas.

**Proteose-peptone acid agar slants.*—Good growth; usually without gas in 3 days.

Stock-culture agar slants.—Slightly heavier growth than on nutrient agar; no gas in 3 days.

Glucose-nitrate agar slants.—Usually good growth. *Variation:* Scant, if any, growth.

**Hydrolysis of starch.*—Positive.

**Production of acetlymethylcarbinol.*—Positive (incubation temperature 32° C.).

pH of glucose broth.—Variable; 4.8 to 7.2.

Fermentation tests.—Acid and usually gas and gum from arabinose, xylose, rhamnose, glucose, sucrose, lactose, mannitol, and sorbitol. (The production of gas was best demonstrated by growing the cultures on the following mediums: Nutrient agar with 1 percent potato starch; potato plugs; and wheat mash as used in the test for crystalline dextrins.)

**Crystalline dextrins.*—Negative.

Hydrolysis of gelatin.—Positive; usually wide zone of hydrolysis. (With widely spreading cultures the zone of hydrolysis sometimes extended only slightly beyond the limits of growth.)

Hydrolysis of casein.—Positive.

Production of indole.—Negative.

Reduction of nitrate to nitrite.—Positive.

Anaerobic production of gas from nitrate.—Negative.

Anaerobic growth in glucose broth.—Positive; vigorous production of gas.

Reduction of methylene blue.—Positive; no reoxidation in 21 days.

Temperatures for growth.—Good growth at 28° to 35° C.; maximum temperature for majority of strains 40°; no growth at 45°.

TYPE CULTURES STUDIED

The following type cultures of *Bacillus polymyxa* were received.

Bacillus polymyxa

No. 279, from Christensen, Difco Labs., 1936, No. 8277.
No. 354, from McCoy, Wis. Univ., 1937.
No. 1105, from ATCC, 1942, No. 842; Stahly; Kluyver.
No. 1238, from NCTC, 1947, No. 1380; Kluyver.
Nos. 1312–1314, from Francis (*54*), Wellcome Res. Labs., England, 1950. Nos. CN2366 (host strain of type C phage), CN1756 (resistant to type C phage), and CN2401 (dissociant of CN1756 and sensitive to type C phage).
No. T2010, from Stansly, Amer. Cyanamid Co., 1947, polymyxin strain (*153*).

SYNONYMY

The following five strains that apparently conformed to their original descriptions were identified as *Bacillus polymyxa.*

Bacillus aerosporus Greer, Jour. Infect. Dis. 42: 508. 1928.

From NCTC, 1947, No. 4747; Porter .... *B. Polymyxa* No. 1237

Bacillus asterosporus (Meyer) Migula, Syst. der Bakt., p. 528. 1900.
Astasia asterosporus A. Meyer, Flora 84: 185. 1897.

	B. polymyxa No.
From Kral, Vienna, 1921	251
From Christensen, Difco Labs., 1936, No. 8278	280
From Porter, Iowa Univ., 1940; Bredemann	812
From Porter, 1940; Meyer	813

All the above strains were studied in detail and were not distinguishable from *B. polymyxa,* thus confirming the observations of Porter, McCleskey, and Levine (*126*).

IDENTIFICATION OF UNNAMED CULTURES

The following unnamed cultures were identified as *Bacillus polymyxa.*

Unnamed cultures	*B. polymyxa* No.
From Reuszer, USDA, 1946	1181–1183
From Tidwell, Hawaii Univ., 1948	1257
From Delaporte, Lab. Cytol. Vég., Paris, 1949, Nos. 10A6, 11B1, and 11B2	1303–1305

Five isolations from soil were made by the writers and identified as *Bacillus polymyxa* (Nos. 252, 293, 297, 391, and 394).

Bacillus macerans

Bacillus macerans Schardinger, Centbl. f. Bakt. [etc.] Abt. 2, 14: 772. 1905.

Rottebazillus 1, Schardinger, Wien. Klin. Wchnschr. 17: 207. 1904.

Owing to its ability to produce acetone, ethyl alcohol, and gas from carbohydrates and its association with the process of retting, *Bacillus macerans* has been studied extensively. Porter, McCleskey, and Levine (*126*) thoroughly reviewed the literature on this species and it will not be repeated here. They pointed out the relationship of *B. macerans* to *B. polymyxa* and gave the characters by which the two could be separated. Biotin and aneurin (thiamine) were reported by Knight and Proom (*84*) as necessary for growth in an ammonia basal medium, in contradistinction to *B. polymyxa,* which required only biotin. The egg-yolk (lecithinase) reaction was of less value, being negative according to Colmer (*34*) and McGaughey and Chu (*110*) and faint and restricted according to Knight and Proom (*84*).

In 1946 (*147*) the close relation of *B. macerans* to *B. circulans* was also observed. *Bacillus circulans* did not produce gas and, as far as was known, neither acetone nor alcohol. The formation of crystalline dextrins by *B. macerans* was thought to be a distinguishing character of the species, but subsequent work has revealed that certain strains of *B. circulans* were also able to change starch into crystalline dextrins. The latter strains, therefore, differed from *B. macerans* only in the production of gas and, perhaps, of acetone and alcohol. The writers are extremely reluctant to divide species by only one character but, at present, have no alternative. It is hoped that further study will disclose other differentiating characteristics or provide proof of a varietal relationship.

The following description of *B. macerans* was based on the study of 20 strains, 10 of which were type cultures, 6 bore names in synonymy, 2 were received unnamed, and 2 were isolated from soil by the writers.

CHARACTERS

**Vegetative rods.*—0.5μ to 0.7μ by 2.5μ to 5.0μ; not in chains; motile; Gram-variable.

On glucose-nutrient agar the cells were wider and longer than on nutrient agar and contained a few small fat globules.

**Sporangia.*—Definitely swollen; clavate.

**Spores.*—1.0μ to 1.5μ by 1.2μ to 2.5μ; oval; subterminal to terminal; spore wall thick and stainable.

Colonies.—Thin; transparent to whitish; irregular; spreading; or small and compact in the rough stage.

Nutrient agar slants.—Growth thin; restricted or spreading; inconspicuous to whitish.

Nutrient broth.—Turbidity light, uniform to granular; with or without flocculent sediment.

NaCl broth.—No growth in 5-percent concentration.

Glucose-nutrient agar slants.—Growth heavier than on nutrient agar, usually with the formation of gas.

Utilization of citrate.—Negative.

**Potato.*—Inconspicuous or scant growth with production of much gas and decomposition of the potato. *Variation:* No growth by 3 strains.

Soybean agar slants.—Growth usually as good as, or better than, on nutrient agar, sometimes with gas.

**Proteose-peptone acid agar slants.*—No growth. *Variation:* Scant growth by 1 strain.

Stock-culture agar slants.—Growth generally better than on nutrient agar; usually no gas.

Glucose-nitrate agar slants.—Very scant, if any, growth.

**Hydrolysis of starch.*—Positive.

**Production of acetylmethylcarbinol.*—Negative (incubation temperature 32° C.).

pH of glucose broth.—5.0 or lower.

Fermentation tests.—Acid, usually with gas, from arabinose, rhamnose, xylose, glucose, sucrose, lactose, mannitol, and sorbitol. (The production of gas was best demonstrated by growing the cultures at 37° C. on the following mediums: Nutrient agar containing 1 percent potato starch; potato plugs; and wheat mash as used in the test for crystalline dextrins.)

**Crystalline dextrins.*—Positive (pl. 3, *E*).

Hydrolysis of gelatin.—Positive; fair to good zone of hydrolysis.

Hydrolysis of casein.—Very slight, if any clearing.

Production of indole.—Negative.

Reduction of nitrate to nitrite.—Positive.

Anaerobic production of gas from nitrate.—Negative. *Variation:* One strain positive.

Anaerobic growth in glucose broth.—Usually positive with formation of gas. (Neutral broth was better than the usual alkaline broth.)

Reduction of methylene blue.—Positive; complete reoxidization in 21 days. *Variation:* Three strains not reoxidized.

Temperatures for growth.—Good growth 28° to 40° C.; maximum temperature for growth 45° to 50°. *Variations:* No growth at 40°; or growth at 54°.

TYPE CULTURES STUDIED

The following authentic cultures of *Bacillus macerans* were obtained.

Bacillus macerans

No. 277, from Christensen, Difco Labs., 1936, No. 8275.
No. 888, from Porter, Iowa Univ., 1940; Schardinger.
No. 1093, from ATCC, 1944, No. 843; Tilden; Kluyver.
Nos. 1095 and 1096, from ATCC, 1944, Nos. 8509 and 8510; Tilden Nos. 18 and 2037.
Nos. 1097–1101, from ATCC, 1944, Nos. 8513–8517; Tilden Nos. 577, 588, 573, 585, and 583; Levine.

SYNONYMY

Named cultures that apparently conformed to their descriptions were identified by the writers as *Bacillus macerans.*

Bacillus acetoethylicum Northrup, Ashe, and Senior, Jour. Biol. Chem. 39: 1. 1919.

From Christensen, Difco Labs., 1936, No. 8276 .... *B. macerans* No. 278

Bacillus betanigrificans Cameron, Esty, and Williams, Food Res. 1: 73. 1936.

	B. macerans No.
From Porter, Iowa Univ., 1937; Cameron	646
From Cameron, NCA, 1937	649

The blackening of canned beets was shown by Cameron and others to depend on the action of this species in the presence of an abnormally high content of iron in the beet juice. Metallic iron accelerated the growth of the organism, which, in turn, increased the amount of iron in solution and raised the pH.

When these cultures were first studied by Clark and Smith (*31, p. 282*) a brownish to black pigment was formed in the presence of metallic iron. Since 1942, however, no blackening has been obtained either on artificial mediums or on beets in the presence of metallic iron. In the interval between tests the cultures were maintained on nutrient agar containing 0.5 percent of starch and kept in a refrigerator. Having lost its original specific characteristic, this species could not be differentiated from *B. macerans.* This situation might be called analogous to that of *B. subtilis* and its black varieties and the original organism might be recognized as *B. macerans* var. *niger.* The writers do not recommend this, unless further study of many more strains should warrant such a separation.

Bacillus schuylkilliensis Eisenberg, Jour. Amer. Waterworks Assn. 34: 365. 1942.

From Eisenberg, Ozone Processes, Inc., Philadelphia, 1943, No. 20 .... *B. macerans* No. 1030

In describing this species, Eisenberg stated, "While the organism possesses some of the properties of *A. macerans* and some peculiar to *A. polymyxa,* it cannot be classified as either of the two." Using many more characters than Eisenberg, the writers could not differentiate this culture from *B. macerans,* due allowance being taken for normal physiological variations.

From Eisenberg, 1943, No. 16. See *B. circulans* No. 1029A and *B. brevis* No. 1029B.

Bacillus soli Alarie and Gray, Canadian Jour. Res., C, 25: 228. 1947.

From Gray, MacDonald Col., Quebec, 1948 .... *B. macerans* No. 1252

Except for its preference for a somewhat lower incubation temperature, this organism could not be distinguished from *B. macerans.* The ability to decompose the binding of filter paper fibers in broth culture observed by Alarie and Gray has also been noted by the writers in typical strains of *B. macerans* and *B. polymyxa.*

Bacillus vagans Alarie and Gray, Canadian Jour. Res., C, 25: 228. 1947.

From Gray, 1948 .... *B. macerans* No. 1255

Gas production by this strain was confined to mediums containing starch. In all other respects it was typical for *B. macerans* and was, therefore, regarded as a weakened or aberrant strain.

IDENTIFICATION OF UNNAMED CULTURES

The following cultures were identified as *Bacillus macerans.*

Unnamed cultures

From Bohrer, NCA:	*B. macerans* No.
1944, No. M29	T40
1947, No. 7468	T133

Two strains, Nos. 373 and 374, were isolated from soil by the writers. In 1946 (*147*) they were classified as *B. macerans-B. circulans* intermediates, mainly because no crystalline dextrins were observed by the method employed. A reexamination of the cultures using the method given above (p. 42) resulted in a strong positive test for crystalline dextrins and a reassignment of the strains to *B. macerans.*

Bacillus stearothermophilus

Bacillus stearothermophilus Donk, Jour. Bact. 5: 373. 1920.

From 1944 to 1949 a collection of 216 strains of aerobic, thermophilic, sporeforming bacteria was assembled (*65*) after requests were made to many laboratories in this country and Europe. Only 33 named cultures, bearing 7 different names, were received; the rest were isolations made by other investigators from canned foods, milk, and compost or by the writers from soil, silage, milk, and milk products. With 1 exception (*Bacillus thermophilus,* a typical strain of *B. pumilus* No. 84) the 33 named cultures could be separated into only 2 distinct groups. The earliest named and described species in 1 group was *B. stearothermophilus* Donk and in the other *B. coagulans* Hammer already characterized in group 1. The literature was carefully searched for accounts of thermophilic aerobic sporeformers not present in this collection. The descriptions found, however, were inadequate and the original cultures unrecognizable. With none of the strains predating *B. stearothermophilus* at hand for comparative purposes, there was no choice but to assign this cumbersome name to the species.

Of the unnamed cultures supplied by other laboratories in response to requests for thermophilic strains, 39 percent belonged to *B. subtilis, B. pumilus, B. circulans,* or to species with even lower temperature ranges of growth. The remaining 61 percent and the isolates obtained by the writers surprisingly proved to be either *B. stearothermophilus* or *B. coagulans.*

The following characterization of *B. stearothermophilus* was based on a study of 98 cultures, of which 6 were type cultures, 1 bore a name in synonymy, 2 were assigned to the species, 72 were received unnamed, and 17 were isolated by the writers; all cultures being incubated at 45° to 50° C. during the observation, unless otherwise noted.

CHARACTERS

**Vegetative rods.*—0.9μ to 1.0μ by 2.5μ to 3.5μ; motile; Gram-variable. *Variations:* 0.6μ by 2.0μ to 5.0μ or filaments.

**Sporangia.*—Definitely swollen; racket-shaped. (See pl. 4, *B.*)

**Spores.*—1.0μ to 1.2μ by 1.5μ to 2.2μ; characteristically variable in size; oval; terminal to subterminal; spore wall thick and stainable.

Colonies.—Not distinctive; pinpoint to small; round to irregular; translucent to opaque; rough to smooth.

Nutrient agar slants.—Growth variable; ranging from thin, scant, rough, and nonspreading to good, opaque, smooth, and spreading.

Nutrient broth.—Turbidity usually uniform.

NaCl broth.—Scant, if any, growth in 3-percent concentration of NaCl.

Glucose-nutrient agar slants.—Growth usually less than that on nutrient agar.

Utilization of citrate.—Usually negative.

Potato.—Scant, if any, growth.

**Soybean agar slants.*—Growth, if any, less than that on nutrient agar.

**Proteose-peptone acid agar slants.*—No growth.

Stock-culture agar slants.—Growth usually less than on nutrient agar.

Glucose-asparagine agar slants.—Growth variable.

Tomato-yeast milk.—Coagulation variable.[13]

**Hydrolysis of starch.*—Positive.

**Production of acetylmethylcarbinol.*—Negative (incubation temperature 45° to 50° C.).

pH of glucose broth.—Variable; from 4.5 to 6.6.

Fermentation tests.—Usually acid without gas from glucose; acid variable from arabinose and xylose. With organic nitrogen, acid from glucose; acid variable from arabinose and xylose.

**Hydrolysis of gelatin.*—Positive; wide zone of hydrolysis. *Variation:* Seven strains weakly positive.

Hydrolysis of casein.—Variable.

Reduction of nitrate to nitrite.—Positive. *Variation:* Ten strains negative.

Anaerobic production of gas from nitrate.—Negative. *Variation:* Four strains positive.

Anaerobic growth in glucose broth.—Usually positive.

**Temperatures for growth.*—Good growth from 50° to 65° C.; variable at 70° and 37°; no growth at 28°. (Growth at the high temperatures was apparently a stable character of the species, because strains growing at 65° and 70° when first examined still grew at these temperatures after 4 years' storage at 28°. At the lower temperatures, 6 failed to grow at 45°; 14 at 37°; 77 at 33°; and all failed at 28° (table 14). After 4 years' storage, however, many strains grew at somewhat lower temperatures than at the beginning. As a result, growth in a water bath held at 65° was considered a fairly reliable character of the species, whereas growth at the lower temperatures was too variable to be of use.)

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[13] Gordon and Smith (*65*) reported that this medium was not coagulated in 3 days at 45° to 50° C. Since then, Stark and Tetrault, Purdue University, have found coagulation by all of their cultures in 3 to 5 days at 65° (*154a*). A hurried reexamination of the writers' cultures showed about 60 percent positive, using dried skim milk and tomato juice in making the medium. The nature of the ingredients used may be a factor in the coagulation of this medium.

TYPE CULTURES STUDIED

The following cultures were received and examined.

Bacillus stearothermophilus

Nos. T17 and T19, from Curran, USDA, 1944; NCA Nos. 1518 and 1503.
No. T85, from ATCC, 1945, No. 7954; NCA No. 1503.
Nos. T91 and T92, from Yessair, NCA, 1946, Nos. 1503 and 1518.
No. T2004, from Brooks, N. Y. [Geneva] Agr. Expt. Sta., 1947; Amer. Can Co., strain CDPI.

SYNONYMY

The following named culture was identified as *Bacillus stearothermophilus.*

Bacillus kaustophilus Prickett, N. Y. [Geneva] Agr. Expt. Sta. Tech. Bul. 147: 38. 1928.

From ATCC, 1944, No. 8005; Yale No. BD53;
Prickett .... *B. stearothermophilus* No. T81

Prickett's account of his organism generally applied to culture No. T81 except for the latter's inability to coagulate litmus milk.

ASSIGNMENT OF NAMED CULTURES

The following cultures were not recognizable from their original description and were identified as *Bacillus stearothermophilus.*

Bacillus calidolactis Hussong and Hammer, Jour. Bact. 15: 179. 1928.

From Curran, USDA, 1944, unnumbered and No. 11;
Iowa State Col. .... *B. stearothermophilus* Nos. T15 and T16

See discussion of this species under the assigned cultures of *B. coagulans.*

IDENTIFICATION OF UNNAMED CULTURES

The following cultures were received without names and were identified as *Bacillus stearothermophilus.*

Unnamed cultures	*B. stearothermophilus* No.
From Curran, USDA, 1944, Nos. 26 and 7028; NCA	T18 and T20
From Pilcher, Amer. Can Co., 1944, Nos. 7B, 21Y, unnumbered, 26A, 26B, and 36A	T28–T33
From Speck, Sealtest Res. Labs., Baltimore, 1944, Nos. TNA25, TH63, TH54, TNA1, and LT1	T34–T37 and T39
From Bohrer, NCA, 1944, Nos. T38, T28, T30, T34, 1792, and T9	T53 and T59–T63
From Bohrer, 1944, Nos. T6, 4298, T37, Avon X–1, 2116, 4109, and T36	T64–T70
From Bohrer, 1944, Nos. T33, T13, 1356, 1549, 1805, 4172, and 4103	T71–T77
From Bohrer, 1944, Nos. Avon M–1, 4208, 2027, and HB	T78–T80 and T82
From Bohrer, 1947, Nos. 1373, 1820, 2548–11, 11555, and 1168	T117, T127, T132, T137, and T141
From Bohrer, 1947, Nos. C–1194, C–1225-3, C–1235–1, C–1235–4, and C–1235–5	T146–T150
From Webley, Aberdeen Univ., Scotland, 1947, strains ATT, BTT, DTT, HTT, JTT, KTT, LTT, and NTT (*175*)	T158–T160 and T164–T168
From Curran, 1947; Amer. Can Co., Nos. C1P4 and C2P3	T188 and T189
From Bohrer, 1947, Nos. C–1889, C–2263–1, C–2263–2, and C–2263–3	T194–T197

From Cleverdon, Maryland Univ., 1948; NCA Nos. 10, 26, 1492, and 2156 .... T2024–T2027
From Olsen, Sugar Refinery Lab., Copenhagen, 1949, Nos. B1, B2, B3, 4B, 5B, B–0, and M1 .... T2037–T2039 and T2041–T2044

Seventeen cultures were isolated by the writers from milk (Nos. T86–T90) and soil (Nos. T93–T95, T106–T110, and T152–T155).

Bacillus circulans

Bacillus circulans Jordan, emend. Ford, Jour. Bact. 1: 519. 1916.
Bacillus circulans Jordan, Mass. State Bd. Health, Expt. Invest., pt. 2, p. 831. 1890.

Bacillus circulans was introduced in 1890 by Jordan and named because of the rotary motion of the mass of cells inside its young colonies. In 1916 Ford isolated a culture that he believed to be the same as Jordan's, but a scrutiny of the characteristics as given shows some discrepancies. The rods, according to Jordan, were 1.0μ by 2.0μ to 5.0μ, with small, oval, terminal spores of about the same diameter as the rods; gelatin was liquefied. On the other hand, Ford described his organism as 0.5μ by 2.5μ to 4.0μ, with spores measuring 0.75μ by 1.25μ; the sporangium, therefore, was definitely bulged; gelatin was not liquefied.

When observed by the writers, Ford's culture still showed a rotary motion within the colonies, and if the surface of an agar plate was dried before use micro-colonies formed at the point of inoculation, rotated to the right or left, and moved out over the surface (*146*). Although Ford failed to see any liquefaction of gelatin, it was hydrolyzed by his culture if tested by the Frazier method (*56*). The writers believe, therefore, that Ford's culture, the only historical strain of this species available, may be accepted as authentic.

In 1938 Gibson and Topping (*64*) referred to the *B. circulans* group as a complex "exhibiting variations in several directions." The writers have certainly found this description applicable, because *B. circulans* has been the most difficult to characterize of all the species of the genus. As represented in the writers' collection, the complex was composed of aggregates of cultures that resembled in some respects certain other closely related species. Some of the cultures, for example, formed crystalline dextrins formerly thought (*147*) to be a unique character of *B. macerans;* some resembled *B. polymyxa* in slime formation or in casein hydrolysis, or both; some, like *B. stearothermophilus,* were inhibited by glucose; and others grew at fairly high temperatures. Variations in other characters not so closely associated with other species of group 2 occurred frequently. Unfortunately, no correlation could be found among these variations that would separate the cultures into distinct groups. The present status of *B. circulans* is, therefore, extremely unsatisfactory and offers a challenge for further investigation.

Knight and Proom (*84*) also noted the close relationship between *B. circulans, B. macerans,* and *B. alvei* and isolated several intermediate types. In a study of 18 strains, classified as *B. circulans,* 12 grew in a casein basal medium plus biotin and aneurin, whereas the growth of the remaining 6 was irregular and could not be improved by the addition of several other metabolites. Colmer (*34*), McGaughey and Chu (*110*), and Knight and Proom (*84*) recorded a negative egg-yolk (lecithinase) reaction with a total of 28 strains.

The following description of *Bacillus circulans* was based on the examination of 89 cultures, of which 7 were type cultures, 7 bore names in synonymy, 14 had other names, 20 were received unnamed, and 41 were isolations.

CHARACTERS

**Vegetative rods.*—0.5μ to 0.7μ by 2.0μ to 5.0μ; some slightly bent; ends rounded or pointed; usually not in chains; usually actively motile; Gram-negative. *Variations:* 0.4μ to 0.9μ by 1.5μ to 5.0μ; with metachromatic granules; or encapsulated.

On glucose-nutrient agar the cells were usually wider and longer than on nutrient agar and contained small fat globules.

**Sporangia.*—Definitely swollen; clavate.

**Spores.*—0.8μ to 1.4μ by 1.1μ to 2.4μ; oval; terminal to subterminal; spore wall thick and stainable (pl. 4, *C*). *Variations:* Kidney-shaped; cylindrical; lateral; central; or thin-walled.

Colonies.—Thin; spreading; translucent to transparent, sometimes barely visible; or small to medium in size; opaque; entire.

Giant colonies.—Sometimes motile micro-colonies rotating from point of inoculation; eventually covering entire plate. (Poured agar plates for this demonstration (*146*) were allowed to stand at room temperature for 2 to 3 days in order to dry the surface before use.)

Nutrient agar slants.—Growth scant; thin; spreading; often indistinct. *Variations:* Moderate; entire; dense; or coherent.

Nutrient broth.—Turbidity light to fair; flocculent to slimy sediment. *Variation:* No growth by a few strains.

NaCl broth.—No growth by some strains in 2-percent concentration of NaCl; growth by others in 5-percent; no growth in 7-percent.

Glucose-nutrient agar slants.—Growth usually heavier than on nutrient agar. *Variations:* Very gummy; opaque; or less growth than on nutrient agar.

Beef-extract agar slants.—Sporulation usually better than on nutrient agar.

Utilization of citrate.—Usually negative.

Potato.—Usually no visible growth. *Variations:* Growth scant to abundant; yellowish to brownish; or gummy.

Soybean agar slants.—Growth, if any, usually less than on nutrient agar.

Proteose-peptone acid agar slants.—Usually no growth.

**Hydrolysis of starch.*—Positive; usually wide zone of hydrolysis.

**Production of acetylmethylcarbinol.*—Negative (incubation temperature 32° C.).

pH of glucose broth.—Usually less than 5.5.

Fermentation tests.—Acid without gas from glucose; usually acid from arabinose, xylose, and sucrose.

Crystalline dextrins.—Negative. *Variation:* Twenty-five percent of the strains positive.

Hydrolysis of gelatin.—Usually positive.

Hydrolysis of casein.—Scant, if any, hydrolysis. *Variation:* Fair to good hydrolysis by 10 strains.

**Production of indole.*—Negative.

Urease.—Usually negative.

Reduction of nitrate to nitrite.—Variable.

Anaerobic production of gas from nitrate.—Usually negative.

Anaerobic growth in glucose broth.—Positive; pH 4.8 to 6.0 at 14 days. *Variation:* About 35 percent negative.

Reduction of methylene blue.—Positive; usually reoxidized in a few days. *Variations:* No growth; or not reoxidized.

Temperatures for growth.—Good growth at 28° to 33° C.; maximum temperature for growth usually 40° to 45°; *Variations:* Growth at 50° to 55° by a few strains; or scant growth at 28°.

TYPE CULTURES STUDIED

The following authentic cultures of *Bacillus circulans* were obtained.

Bacillus circulans

No. 358, from AMNH, 1925; Ford.
No. 726, from ATCC, 1939, No. 4513; Ford No. 26.
No. 831, from Porter, Iowa Univ., 1940; NCTC No. 2601; Ford No. 26.
No. 1136, from Randall, Food and Drug Admin., 1946, for assay of streptomycin (*127*).
No. 1280, from Henriksen, Bakt. Inst., Oslo, 1949.
Nos. 1281 and 1282, from Henriksen, 1949; Turner and Eales, strains A and B (*169*).

From Steinhaus, USPHS, 1942, No. 66. See *B. sphaericus* No. 1023.

SYNONYMY

Named cultures that apparently conformed to their descriptions were identified as *Bacillus circulans* as follows.

Bacillus amylolyticus Kellerman and McBeth, Centbl. f. Bakt. [etc.] Abt. 2, 34: 485. 1912.

From Kellerman, USDA, 1912 .... *B. circulans* No. 290

Kellerman and McBeth isolated and named this organism because of its strong action on starch. At first it dissolved precipitated cellulose (hydroxycellulose) with ammonium salts as the source of nitrogen but soon lost that property. If grown in nutrient broth containing strips of filter paper extending above the surface, it has usually digested the adhesive substances of the paper, allowing the cellulose fibers to separate.

In 1946 (*147*) culture No. 290 was listed as an intermediate between *B. macerans* and *B. circulans* because of its formation of crystalline dextrins. A change in the method for the better detection of crystalline dextrins, however, revealed that 25 percent of the strains of *B. circulans* were positive. Culture No. 290 could, therefore, no longer be regarded as an intermediate.

Bacillus closteroides Gray and Thornton, Zentbl. f. Bakt. [etc.] Abt. 2, 73: 93. 1928.

From Porter, Iowa Univ., 1940; Thornton..........*B. circulans* No. 832

This culture agreed with its original description and with that of *B. circulans*, with the possible exception of the utilization of phenol, a property not investigated in the present work. Judging from the variations exhibited by the strains of this complex, it is highly improbable that such a character as the utilization of phenol would be stable or of such importance as to warrant specific designation.

Bacillus effluens Alarie and Gray, Canadian Jour. Res., C, 25: 228. 1947.

From Gray, MacDonald Col., Quebec, 1948........*B. circulans* No. 1250

Culture No. 1250 was similar to No. 760 (received as *B. krzemieniewski* and listed below) in its production of a great deal of gum from carbohydrates and in its failure to grow anaerobically in glucose broth. It differed from No. 760, however, in its inability to hydrolyze casein.

Bacillus kellermanii Alarie and Gray, Canadian Jour. Res., C, 25: 228. 1947.

From Gray, 1948 ....*B. circulans* No. 1251

This culture was very similar to strain No. 290, received as *B. amylolyticus*.

Bacillus krzemieniewski Kleczkowska, Norman, and Snieszko, Soil Sci. 49: 185. 1940.

From Snieszko, Maine Univ., 1942 ....*B. circulans* No. 760

Isolated from arable soil during soil-fertility tests by the Azotobacter plaque method, this organism was characterized by the presence of capsules and the production of large quantities of gum, especially on mediums containing simple carbohydrates and very low in nitrogen. Although it grew well on Ashby's agar (used for the growth of Azotobacter), nitrogen fixation could not be demonstrated.

Culture No. 760 differed from the majority of strains assigned to the *B. circulans* complex in being encapsulated, forming a gummy mass on carbohydrate mediums, in a more active decomposition of casein, and in its failure to grow anaerobically in glucose broth. At first it was thought likely that strain No. 760 represented a distinct variety. Subseqent work, however, revealed that certain other strains possessed these same characters in varying degrees and that a dividing line could not be drawn.

Bacillus torquens Alarie and Gray, Canadian Jour. Res., C, 25: 228. 1947.

From Gray, 1948, Nos. 2 and 10........*B. circulans* Nos. 1253 and 1254

These cultures differed from the majority of strains of *B. circulans* only in their inability to grow anaerobically in glucose broth and at 37° C. Number 1253 formed only a few spores.

ASSIGNMENT OF OTHER NAMED CULTURES

The following cultures did not conform to, or were not recognizable from, their descriptions and have been identified as *Bacillus circulans*.

Bacillus carotarum

From Porter, Iowa Univ., 1940; Bredemann; Stapp..*B. circulans* No. 826

See synonymy of *B. megaterium*.

Bacillus cohaerens

From Porter:	*B. circulans* No.
1940; Bredemann	838A
1940; Stapp	839A

For other strains bearing this name, see the assigned cultures under *B.*

megaterium. Cultures Nos. 838A and 839A, contaminants of the original cultures, did not grow anaerobically in glucose broth and were able to utilize citrate.

Bacillus consolidus Stührk, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 191. 1935.

From Porter, 1940; Bredemann .... *B. circulans* No. 841

In the above brief account, the spores were reported as round to cylindrical and smaller in diameter than the rods and starch was not hydrolyzed. This description did not apply to culture No. 841.

Bacillus fusiformis

From ATCC:	*B. circulans* No.
1939, No. 61; Ford No. 10	727
1939, No. 4515; Ford No. 24	728

For authentic cultures, see *B. sphaericus*. In 1946 (*147*) culture No. 727 was regarded as a biotype of *B. circulans* because of its formation of acetoin. Subsequently, it was found to be contaminated with *B. pumilus*, which accounted for its abnormal behavior.

Bacillus globigii

From ATCC, 1936, No. 4516; Ford No. 7; Kral.... *B. circulans* No. 313

For a discussion of cultures bearing the above name, see synonymy of *B. licheniformis*.

Bacillus lautus Batchelor, Jour. Bact. 4: 30. 1919.

From Henry, Wash. (State) Univ., 1937, No. 160.. *B. circulans* No. 666

As received, this culture was mixed with *B. cereus* and after purification failed to check with Batchelor's description.

Bacillus nitidus Heigener, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 99. 1935.

From Porter, 1937; Bredemann .... *B. circulans* No. 625

This organism resembled strains Nos. 838A and 839A (received as *B. cohaerens*) in its ability to utilize citrate and failure to grow anaerobically in glucose broth.

Bacillus pabuli Schieblich, Centbl. f. Bakt. [etc.] Abt. 2, 58: 204. 1923.

From Porter, 1940; Schieblich .... *B. circulans* No. 924

Strain No. 924 formed acid readily from carbohydrates, whereas Schieblich's culture was reported as negative in this respect.

Bacillus schuylkilliensis

From Eisenberg, Ozone Processes, Inc., Philadelphia,
1943, No. 16 .... *B. circulans* No. 1029A

Eisenberg's strain No. 16 was separated into No. 1029A and *B. brevis* No. 1029B. Culture No. 1029A conformed to the majority of strains of *B. circulans* except for its formation of crystalline dextrins. For an authentic culture of *B. schuylkilliensis* see the synonymy of *B. macerans*.

Bacillus suprarestistens Stührk, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 185. 1935.

From Porter, 1940; Bredemann.................. *B. circulans* No. 977

This strain was somewhat irregular in that it preferred a temperature of about 40° C. and grew at 52°, which was 5° to 10° higher than the maximum temperature of most other strains. Good growth occurred on potato; citrate was utilized; crystalline dextrins were formed; and there was no anaerobic growth in glucose broth.

Bacillus terminalis Migula, Syst. der Bakt., p. 578. 1900.
B. lactis XII Flügge, Ztschr. f. Hyg. u. Infektionskrank. 17: 296. 1894.

From ATCC, 1939, No. 4530; Ford No. 28 .... *B. circulans* No. 746

Lawrence and Ford (*94, p. 314*) made two isolations that they believed corresponded to this species. They reported alkalinity in broth containing glucose, lactose, and sucrose. Since culture No. 746 formed acid, it is probably not their strain.

Bacillus validus Heigener, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 97. 1935.

From Porter, 1937; Bredemann .... *B. circulans* No. 639

This culture was similar to No. 625 (received as *B. nitidus*) above.

IDENTIFICATION OF UNNAMED CULTURES

The following unnamed cultures were received during the course of this work and identified as *Bacillus circulans.*

Unnamed cultures	*B. circulans* No.
From Russ-Münzer, Deutsches Univ., Prague, 1938, Nos. WI and WII (*135*)	715 and 716
From Myers, Natl. Dairy Labs., Baltimore, 1939, No. RB3A	765
From Townsend and Zuch, Calif. Univ. and NCA, 1943, strain B (*166*)	1061
From Pollard, Tenn. Univ., 1946, strain B	1145
From Reuszer, USDA, 1946	1167–1177
From McLeod, USPHS, 1947, No. M14	1218
From Shaw, N. Y. Dept. of Health, 1949, No. 4204	1275
From McLeod, 1949, No. N–3	1278
From O'Brien, USDA, 1950	1337

Culture No. 765 resembled No. 760 (received as *B. krzemieniewski*) in its production of a great deal of gum from carbohydrates, heavy growth on potato, and hydrolysis of casein. It differed, however, in its ability to grow anaerobically in glucose broth and in its formation of crystalline dextrins. Because of the latter character it was listed in 1946 (*147*) as a *B. macerans*-*B. circulans* intermediate.

Culture No. 1061 resembled Nos. 838A and 839A, received as *B. cohaerens.*

Cultures Nos. 1145 and 1167 to 1177 produced crystalline dextrins, with the exception of Nos. 1170, 1171, and 1172. Number 1172 also differed from the others in its failure to grow anaerobically in glucose broth.

Culture No. 1218 required an incubation temperature of 37° C. for good growth. It was like culture No. 760 (received as *B. krzemieniewski*) in its formation of gum from carbohydrates and hydrolysis of casein. On the other hand, it differed in its formation of crystalline dextrins, anaerobic growth in glucose broth, and failure to attack citrate.

Culture No. 1275 grew well at 28° C., but otherwise resembled No. 1218.

Culture No. 1278 was similar to No. 1218.

The following 41 strains were isolated from soil by the writers and assigned to the *B. circulans* complex: Nos. 291, 292, 294, 295, 359, 375, 381–390, 396–399, 676–679, and 1340–1356.

Bacillus alvei

Bacillus alvei Cheshire and Cheyne, Roy. Micros. Soc. Jour., Ser. 2, 5: 592. 1895.

Bacillus alvei, having first been isolated from hives of honey bees affected with foulbrood, was thought for a time to be the causative agent of the disease. In the original description, Cheshire and Cheyne noted that the spores, which were very large, lay side by side in long rows and that the vegetative cells grew out from the line of inoculation as pointed processes.

In examining cultures of this species inoculated on rather dry nutrient agar plates, Smith and Clark (*146*) observed micro-colonies breaking away from the main portion of the growth and migrating widespread over the surfaces of the plates. These colonies were composed of varying numbers of cells, all in a parallel arrangement and moving together as a unit. As the colonies became larger and the cells older, the forward motion became less and less. Although active motility and motile colonies were characteristic of the species, a nonmotile variant of one of the motile cultures was obtained in the laboratory by colony selection (*29*).

In 1946 (*147*) the writers combined the species *B. para-alvei* with *B. alvei* because no specific differences were found between the two. The morphological differences described by Tarr (*161*) could not be substantiated. Katznelson and Lockhead (*82*), however, reported that in a mixture of amino acids *B. para-alvei* was able to make moderate growth without thiamine, whereas *B. alvei* required thiamine. Neither species could grow in casein hydrolysate medium without thiamine. Knight and Proom (*84*), examining five strains of *B. alvei*, found the nutritional pattern of three strains in accord with that given by Katznelson and Lochhead. The fourth strain required both biotin and thiamine and the fifth had even more complex requirements. Colmer (*34*) noted that the egg-yolk (lecithinase) reaction of one strain of *B. alvei* was negative, and McGaughey and Chu (*110*) obtained the same result with three cultures. Knight and Proom (*84*) however, reported a restricted reaction with four strains.

The following description of *B. alvei* was based on the study of 12 strains; of these 4 were type cultures, 3 were labeled *B. para-alvei*, 2 were received unnamed, and 3 were isolated by the writers.

CHARACTERS

**Vegetative rods.*—0.5μ to 0.8μ by 2.0μ to 5.0μ; frequently side by side in long rows; motile; Gram-variable. *Variation:* Nonmotile and with capsular material.

On glucose-nutrient agar cells contained a few small fat globules.

**Sporangia.*—Distinctly bulged; spindle-shaped to clavate.

**Spores.*—0.8μ to 1.0μ by 1.2μ to 2.0μ; central to terminal; oval; spore wall thick and stainable; free spores frequently in parallel arrangement like that of the rods. (See pl. 4, *D*.)

Colonies.—Thin; smooth; translucent; quickly spreading as a thin layer over entire plate. *Variation:* Round and rather gummy.

**Giant colonies.*—Motile micro-colonies moving in large arcs from point of inoculation; bullet-shaped; plate usually covered in 1 day. (Poured agar plates for this demonstration (*146*) were allowed to stand at room temperature for a couple of days before using in order to dry the surface.)

Nutrient agar slants.—Growth thin; flat; spreading over the surface; migating colonies on the upper, drier part of the slant. *Variation:* Growth thick and rather gummy.

Nutrient broth.—Turbidity light to moderate; uniform.

NaCl broth.—No growth in 5-percent concentration of NaCl.

Glucose-nutrient agar slants.—Growth thinner or thicker than on nutrient agar.

Utilization of citrate.—Negative.

Potato.—Growth, if any, inconspicuous to moderate; spreading; yellowish.

Soybean agar slants.—Growth, if any, scant, thin, and spreading. *Variation:* Abundant, dense, and wrinkled.

Proteose-peptone acid agar slants.—No growth.

Glucose-nitrate agar slants.—Scant, if any, growth.

**Hydrolysis of starch.*—Positive.

**Production of acetylmethylcarbinol.*—Positive. (One strain, No. 684, required 16 to 20 days' incubation at 32° C.)

pH of glucose broth.—4.8 to 5.6. *Variation:* Two strains, Nos. 684 and 811, pH 6.8 and 7.4, respectively.

**Fermentation tests.*—Acid without gas from glucose; usually acid from sucrose; usually no acid from lactose and mannitol; no acid from xylose and arabinose.

Hydrolysis of gelatin.—Positive.

Hydrolysis of casein.—Positive.

**Production of indole.*[14]—Positive.

Urease.—Negative.

Reduction of nitrate to nitrite.—Negative.

Anaerobic production of gas from nitrate.—Negative.

Anaerobic growth in glucose broth.—Positive, often with few bubbles of gas; pH 4.6 to 5.6 after 14 days.

Reduction of methylene blue.—Positive; reoxidation variable after 21 days.

Temperatures for growth.—Good growth at 28° to 37° C.; maximum temperature for growth 43° to 45°.

TYPE CULTURES STUDIED

The following type cultures of *Bacillus alvei* were obtained and studied.

Bacillus alvei

No. 662, from Lochhead, Dept. of Agr., Ottawa, 1937.
Nos. 683 and 684, from Hambleton, USDA, 1937, strains N. C. and Ga. No. 20443.
No. 811, from Porter, Iowa Univ., 1940; Lochhead No. 127.
From Porter. 1940; Bredemann; Neide. See *B. sphaericus* No. 810.

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[14] In 1947 S. R. Dutky, USDA, Moorestown, N. J., after examining 10 cultures supplied by the writers, found them all positive for indole and suggested that this test might be useful for the identification of the species.

SYNONYMY

Named cultures that conformed to their original description were identified by the writers as *Bacillus alvei*, as follows.

Bacillus para-alvei Burnside, Amer. Bee Jour. 72: 433. 1932.

	B. alvei No.
From Hambleton, USDA, 1937, Nos. 20485–1 and 20485–2	685 and 686
From Lochhead, Dept. of Agr., Ottawa, 1946, No. 316; Hambleton, strain CHH	1141

This organism was isolated from honey bees affected with parafoulbrood. The bacterial forms found in larvae resembled those observed in European foulbrood, indicating a close relationship. The distinguishing feature of the bacillus appeared to have been its "marked variability and pleomorphism, depending to some extent on culture medium and temperature but perhaps in greater degree upon its physiological condition" (*19, p. 582*). Coccoid and streptococcic forms were isolated as variants, as well as a small granular rod. The cultures studied by the writers failed to show such morphological variation and agreed with the other strains of *B. alvei*, even producing motile microcolonies.

IDENTIFICATION OF UNNAMED CULTURES

The following unnamed strains were identified as *Bacillus alvei.*

Unnamed cultures	*B. alvei* No.
From Reuszer, USDA, 1946	1180
From Delaporte, Cold Spring Harbor, 1949	1306

Three strains (Nos. 395, 680, and 750) were isolated from soil by the writers.

Bacillus laterosporus

Bacillus laterosporus Laubach, Jour. Bact. 1: 511. 1916.

In 1912 White (*179*) gave the name *Bacillus orpheus* to a motile sporeforming organism found in the foulbrood of honey bees and stated that its description would be given later. This was done in 1917 by McCray (*109*). In the meantime, Laubach, in 1916, described an organism isolated from water, which he called *B. laterosporus* because the spore appeared at the side of the rod, i.e., eccentrically (pl. 4, *E*). As the spore matured a remnant of the sporangium adhered to one side. This morphological feature seemed to be quite constant. In the present work, however, certain other closely related species, especially *B. brevis* and *B. circulans,* at times formed spores showing this character, but the number of such spores in a smear was never very large.

The original descriptions of both *B. laterosporus* and *B. orpheus* were quite complete and leave little doubt that they were identical. The mere naming of a micro-organism, however, does not constitute effective publication and is not valid (*16, p. 295*). The name *B. orpheus* should, therefore, be discarded and the name *B. laterosporus* used to designate this species.

The following description of *B. laterosporus* was based on the study of 13 strains, of which 5 were type cultures, 3 bore names in synonymy, 3 were received unnamed, and 2 were isolated by the writers.

CHARACTERS

**Vegetative rods.*—0.5μ to 0.8μ by 2.0μ to 5.0μ; ends poorly rounded or pointed; motile; Gram-variable. *Variations:* Smaller or larger in diameter.

**Sporangia.*—Definitely bulged; spindle-shaped.

**Spores.*—1.0μ to 1.3μ by 1.2μ to 1.5μ; oval; central; lateral; spore wall thick and stainable; remnants of sporangium adhering to mature spore thicker on one side of the spore than on the other. (See pl. 4, *E*.)

Colonies.—Thin; translucent; spreading; irregular. *Variations:* Small; round; rough; or opaque.

Nutrient agar slants.—Growth thin; flat; spreading; translucent. *Variations:* Abundant; restricted; or opaque.

Nutrient broth.—Turbidity uniform to granular.

NaCl broth.—Usually growth in 2-percent concentration of NaCl; no growth in 5-percent.

Glucose-nutrient agar slants.—Growth heavier than on nutrient agar; dense; wrinkled.

**Utilization of citrate.*—Negative.

Potato.—Growth scant to abundant; spreading; gray, pink, or brown.

Soybean agar slants.—Growth variable.

**Hydrolysis of starch.*—Negative.

**Production of acetylmethylcarbinol.*—Negative (incubation temperature 32° C.).

**pH of glucose broth.*—6.0 to 7.4 after 7 days.

**Fermentation tests.*—Acid without gas from glucose and mannitol; usually acid from sucrose; usually no acid from lactose; no acid from arabinose and xylose.

Hydrolysis of gelatin.—Positive. *Variation:* Two strains (Nos. 1338 and 1339) negative.

Hydrolysis of casein.—Positive; usually wide zone of hydrolysis.

**Production of indole.*—Positive. *Variation:* Two strains (Nos. 681 and 682) negative.

Urease.—Negative.

Reduction of nitrate to nitrite.—Positive.

Anaerobic production of gas from nitrate.—Usually positive, showing a few bubbles of gas.

Anaerobic growth in glucose broth.—Positive; pH 4.8 to 5.8.

Temperatures for growth.—Good growth at 28° to 37° C.; no growth at 45°.

TYPE CULTURES STUDIED

The following type cultures of *Bacillus laterosporus* were obtained and studied.

Bacillus laterosporus

No. 314, from ATCC, 1936, No. 64; AMNH No. 797; Ford No. 6.
No. 340, from NCTC, 1937, No. 2613; Ford No. 29.
No. 347, from ATCC, 1936, No. 4517; Ford No. 29.

No. 590, from NIH, 1937, Clinical Pathogen Series.
No. 882, from Porter, Iowa Univ., 1940; NCTC No. 2613; Ford No. 29.

It should be noted that Ford's strain No. 29 was obtained from three different sources. Numbers 340 and 882 agreed quite closely, but No. 347, although conforming in the main, was a weaker strain. It was unable to grow on the basal carbohydrate medium and sporulated very sparingly.

From NIH, 1937, Clinical Pathogen Series. See *B. cereus* 589.

SYNONYMY

The following cultures conformed to their original description and were identified as *Bacillus laterosporus.*

Bacillus orpheus White, U. S. Dept. Agr. Bur. Ent. Cir. 157: 3. 1912.
McCray, Jour. Agr. Res. 8: 399. 1917.

	B. laterosporus No.
From Lochhead, Dept. of Agr., Ottawa, 1937	661
From Hambleton, USDA, 1937, Nos. 20485–1 and 20485–2	681 and 682

IDENTIFICATION OF UNNAMED CULTURES

The following strains were received during the course of this work and identified as *Bacillus laterosporus.*

Unnamed cultures	*B. laterosporus* No.
From ATCC, 1944, laboratory contaminant	1111
From Bogusky, USDA, 1948, strains R and S	1267 and 1268

Two strains (Nos. 1338 and 1339) were isolated from soil by the writers.

Bacillus pulvifaciens

Bacillus pulvifaciens Katznelson, Jour. Bact. 59: 153. 1950.

While investigating the cause of death of honey bee larvae, Katznelson observed dry, powdery, light-brown scales that were quite unlike those of European and American foulbrood diseases and which contained pure cultures of a short, Gram-positive, sporulating rod. When first isolated the cultures formed a reddish-brown pigment, but they rapidly dissociated to the colorless stage when cultivated on laboratory mediums. Morphologically and physiologically they were distinguishable from *B. laterosporus* and *B. larvae* and seemed to represent a new species.

The following description was based on the study of three strains kindly supplied by Katznelson (temperature of incubation, 37° C.).

CHARACTERS

**Vegetative rods.*—0.3μ to 0.6μ by 1.5μ to 3.0μ; not in chains; ends rounded; motile; Gram-positive.

On glucose-nutrient agar cells appeared larger than on nutrient agar.

**Sporangia.*—Definitely swollen; spindle-shaped to clavate.

**Spores.*—1.0μ by 1.3μ to 1.5μ; oval; central to subterminal; spore wall thick and stainable. (Sporulation was variable on most mediums; best on potato.)

Colonies on glucose-nutrient agar.—Small; round; smooth; dense; orange to colorless.

Nutrient agar slants.—Growth scant; inconspicuous; restricted; pink to orange, rapidly dissociating to the colorless stage.

Nutrient broth.—Fair, uniform turbidity.

**NaCl broth.*—Growth in 5-percent concentration of NaCl; no growth in 8-percent concentration.

**Glucose-nutrient agar slants.*—Growth dense; restricted to spreading; colorless to reddish; rapidly dissociating to the colorless stage; many needle-, boat-, and bayonnet-shaped microcrystals in growth at 6 days.

Tyrosine agar slants.—Growth and pigment same as on nutrient agar.

Utilization of citrate.—Negative.

**Potato.*—Growth moderate; spreading; thin; orange. (Sporulation and pigmentation have been maintained practically without change for 18 months by transferring to potato every 2 months.)

Soybean agar slants.—Growth scant to fair; pink to orange, rapidly dissociating to the colorless stage; many needlelike microcrystals.

**Hydrolysis of starch.*—Negative.

Production of acetylmethylcarbinol.—Negative (incubation temperature 37° C.).

**pH of glucose broth.*—5.0 to 5.8.

Fermentation tests.—No growth on basal carbohydrate medium.

Hydrolysis of gelatin.—Positive.

Hyrolysis of casein.—Positive; wide zone of clearing after 4 days.

**Production of indole.*—Negative.

Urease.—Negative.

Reduction of nitrate to nitrite.—Positive.

Anaerobic production of gas from nitrate.—Negative.

Anaerobic growth in glucose broth.—Positive; pH 5.2 to 5.6 after 2 weeks.

Reduction of methylene blue.—Positive.

Temperatures for growth.—Good growth at 35° to 45° C.; poor growth and less pigmentation at 28°; no growth at 50°.

TYPE CULTURES STUDIED

The following type cultures of *Bacillus pulvifaciens* were examined.

Bacillus pulvifaciens

Nos. 1283–1285, from Katznelson, Dept. of Agr., Ottawa, 1949, Nos. 113, 221, and 351.

Bacillus brevis

Bacillus brevis (Flügge) Migula, emend. Ford, Jour. Bact. 1: 522. 1916.

B. brevis (Flügge) Migula, Syst. der Bakt., p. 583. 1900.

B. lactis I, Flügge, Ztschr. f. Hyg. u. Infektionskrank. 17: 294. 1894.

In 1900 Migula renamed Flügge's *Bacillus lactis* I, calling it *B. brevis*, but added nothing to the original account. In 1904 Neide (*119*) independently changed the name of Flügge's culture to *B. lactis* and emended its characterization considerably. Cultures of *B. lactis* are still available that conform in general to Neide's description. Such cultures were studied in the present work and were listed in the synonymy of *B. cereus*. So far as the writers are aware, there is no culture available from Migula or Flügge bearing the name *B. brevis*.

Ford (*93, p. 522*) isolated three cultures from soil and called them *B. brevis* because he believed that they fitted Flügge's and Migula's accounts. He described them fully and deposited cultures with the NCTC and ATCC. Unfortunately, the strains from the ATCC did not correspond to Ford's description when examined in the present work, being strains of *B. pumilus*. The culture from the NCTC, on the other hand, did conform and has been accepted as a true representative of Ford's cultures.

Bacillus brevis received a great deal of publicity from the work of Dubos (*49*) and associates on the production and application of antibiotic substances (gramicidin and tyrocidin). His soil bacillus was identified by the writers and placed in their collection as *B. brevis* No. 751. Several stock cultures of the same species from the writers' collection were sent to Dubos and found to produce small quantities of antibiotics but not nearly so much as his isolation.[15] This was expected, as Dubos had specially selected his culture for this particular character over a period of 2 years.

Nine of the 10 cultures of *B. brevis* examined by Knight and Proom (*84*) grew on casein hydrolysate medium without added growth factors. The tenth strain, however, which they recognized as aberrant, required aneurin (thiamine), biotin, and nicotinic acid. Colmer (*34*) and McGaughey and Chu (*110*) reported a negative egg-yolk (lecithinase) reaction for a total of 7 strains and Knight and Proom (*84*) a faint restricted reaction for 9 strains.

The following description of *B. brevis* was based on a study of 71 strains, of which 4 were type cultures, 3 bore names in synonymy, 25 had other names, 33 were received unnamed, and 6 were isolated from soil by the writers.

CHARACTERS

**Vegetative rods.*—0.6μ to 0.9μ by 1.5μ to 4.0μ; ends poorly rounded or pointed; not in chains; motile; protoplasm finely granular; Gram-variable, usually negative. *Variations:* Smaller or larger in diameter; two strains (Nos. 782 and 799) encapsulated.

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[15] Oral communication, 1940.

On glucose-nutrient agar cells were usually larger than on nutrient agar and contained numerous small fat globules.

**Sporangia.*—Definitely bulged; spindle-shaped to clavate.

**Spores.*—1.0μ to 1.3μ by 1.3μ to 2.0μ; oval; central to subterminal; spore wall usually thick and stainable. *Variation:* Lateral. (Approximately 50 percent of the spores of strain No. 1027 resembled those of *B. laterosporus.*)

Colonies.—Thin; smooth; translucent; quickly spreading over entire plate. *Variations:* Small; round; opaque; or nonspreading.

Nutrient agar slants.—Growth thin; smooth; spreading; translucent, becoming opaque and creamy with age. *Variation:* Growth thin and nonspreading.

Nutrient broth.—Turbidity usually heavy; uniform; with or without fragile pellicle.

NaCl broth.—Usually no growth in 2-percent concentration of NaCl.

Glucose-nutrient agar slants.—Growth usually more abundant than on nutrient agar; wrinkled; spreading.

Utilization of citrate.—Variable.

Potato.—Growth scant to moderate; flat; spreading; creamy yellow, pink, or brownish; frequently cannot be distinguished from growth of *B. pumilus.*

Soybean agar slants.—Growth usually abundant; dense; spreading.

**Hydrolysis of starch.*—Negative.

**Production of acetylmethylcarbinol.*—Negative (incubation temperature 32° C.).

**pH of glucose broth.*—8.0 to 8.6.

Fermentation tests.—Acid without gas from glucose and sucrose; usually acid from mannitol; no acid from xylose, arabinose, and lactose. (If a strain of *B. brevis* did not grow on the basal carbohydrate medium, nutrient agar plus the carbohydrates could not be substituted, because of the strong proteolytic activity of the organism.)

Hydrolysis of gelatin.—Positive.

Hyrolysis of casein.—Positive.

**Production of indole.*—Negative.

Urease.—Negative.

Reduction of nitrate to nitrite.—Variable.

Anaerobic production of gas from nitrate.—Negative. *Variation:* Five strains positive (Nos. 606, 948, 1137, 1138, and 1139).

Anaerobic growth in glucose broth.—Negative.

Temperatures for growth.—Good growth at 28° to 40° C.; maximum temperature for growth usually between 45° and 55°.

TYPE CULTURES STUDIED

The following type cultures of *Bacillus brevis* were obtained and studied.

Bacillus brevis

No. 604, from Porter, Iowa Univ., 1937; NCTC No. 2611; Ford No. 27B.
No. 1137, from ATCC, 1946, No. 9999; NCTC No. 7096, gramicidin S strain (*58*).

Nos. 1138 and 1139, from Hegarty, Sharp and Dohme, Inc., 1946; Waksman; Inst. Trop. Med., Moscow, Nos. B1 and B2, gramicidin S strains (*58*).

From ATCC, 1939, No. 18; AMNH; Ford. See *B. pumilus* No. 724.
From ATCC, 1939, No. 4510; Ford No. 27. See *B. pumilus* No. 725.

SYNONYMY

Named cultures that conformed to their descriptions were identified as *Bacillus brevis*, as follows.

Bacillus centrosporus Ford, Jour. Bact. 1: 524. 1916.

From Henry, Wash. (State) Univ., 1937, No. 120......*B. brevis* No. 664

Ford apparently separated this species from *B. brevis* only on the basis of a restricted growth and usually central position of the spore. Culture No. 664, however, with its central to terminal spores and spreading growth, was not distinct from a typical *B. brevis*.

Bacillus hollandicus Stapp, Centbl. f. Bakt. [etc.] Abt. 2, 51: 47. 1920.

From Porter, Iowa Univ.:	*B. brevis* No.
1937; Bredemann	616
1940; Stapp	874

In general, these two cultures agreed with Stapp's description. Certain discrepancies were noted that might be explained by differences in techniques, i.e., a slightly larger diameter of the spores, reduction of methylene blue, and a higher maximum temperature of growth.

From Porter, 1940; Claussen. See *B. cereus* No. 875.

ASSIGNMENT OF OTHER NAMED CULTURES

The following named cultures that did not conform to or were not recognizable from their original description were identified as *Bacillus brevis*.

Bacillus aegyptiacus Werner, Zentbl. f. Bakt. [etc.] Abt. 2, 87: 459. 1933.

From Porter, Iowa Univ., 1940; Bredemann..........*B. brevis* No. 803

Bacillus agri Laubach and Rice, Jour. Bact. 1: 516. 1916.

From Lamanna, Camp Detrick, 1947, No. 13..........*B. brevis* No. 1219

Bacillus alpinus Werner, Zentbl. f. Bakt. [etc.] Abt. 2, 87: 465. 1933.

From Porter, 1937; Bredemann ....*B. brevis* No. 603

Bacillus bellus Heigener, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 96. 1935.

From Porter, 1937; Bredemann ....*B. brevis* No. 605

Bacillus betainovorans Heigener, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 94. 1935.

From Porter, 1937; Bredemann ....*B. brevis* No. 606

Bacillus borstelensis Stührk, Zentbl. f. Bakt [etc.] Abt. 2, 93: 179. 1935.

From Porter, 1940; Bredemann ....*B. brevis* No. 818

Bacillus dentatus Heigener, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 106. 1935.

From Porter, 1937; Bredemann ....*B. brevis* No. 611

Bacillus elegans Heigener, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 103. 1935.

From Porter, 1937; Bredemann ....*B. brevis* No. 612

These eight species were so poorly described by the various workers that it was not possible to say whether or not the cultures represented the original ones.

Bacillus firmus

From Porter:	*B. brevis* No.
1937; Bredemann, strain Rauschen	856
1937; Bredemann, strain Utliberg	857
1937; Bredemann, strain Predigtstuhl	859

Seven cultures bearing this name were received, four of which were considered authentic and are listed under *B. firmus* in group 1.

Bacillus formosus Heigener, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 101. 1935.

From Porter, 1937; Bredemann .... *B. brevis* No. 614

Bacillus granularis Stührk, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 180. 1935.

From Porter, 1940; Bredemann .... *B. brevis* No. 870

Bacillus limnophilus Stührk, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 190. 1935.

From Porter, 1940; Bredemann .... *B. brevis* No. 887

Bacillus maculatus Stührk, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 184. 1935.

From Porter, 1940; Bredemann .... *B. brevis* No. 889

Bacillus montanus Werner, Zentbl. f. Bakt. [etc.] Abt. 2, 87: 458. 1933.

From Porter, 1937; Bredemann .... *B. brevis* No. 622

Bacillus mycoides

From Porter, 1940; Bredemann; Gottheil .... *B. brevis* No. 910

For authentic strains, see *B. cereus* var. *mycoides.*

Bacillus pallidus Heigener, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 98. 1935.

From Porter, 1937; Bredemann .... *B. brevis* No. 628

Bacillus rarus Werner, Zentbl. f. Bakt. [etc.] Abt. 2, 87: 456. 1933.

From Porter, 1937; Bredemann .... *B. brevis* No. 632

Bacillus rufescens Stührk, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 178. 1935.

From Porter, 1940; Bredemann .... *B. brevis* No. 948

This strain produced a reddish-brown discoloration of most mediums, and the pigmentation could be increased to dark red or black by the addition of tyrosine. There is a possibility that culture No. 948 should be considered as a variety of *B. brevis*, but such a decision awaits further information and the isolation of more strains. (Compare with *B. subtilis* var. *niger.*)

Bacillus santiagensis Stührk, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 188. 1935.

From Porter, 1940; Bredemann .... *B. brevis* No. 953

Bacillus schuylkilliensis

From Eisenberg, Ozone Processes, Inc., Philadelphia, 1943,
No. 16 .... *B. brevis* No. 1029B

Culture No. 1029B was separated from No. 1029A, *B. circulans.* For an authentic culture see the synonym of *B. macerans.*

Bacillus segetalis Werner, Zentbl. f. Bakt. [etc.] Abt. 2, 87: 467. 1933.

From Porter, 1937; Bredemann .... *B. brevis* No. 635

Bacillus valinovorans Heigener, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 104. 1935.

From Porter, 1937; Bredemann .... *B. brevis* No. 640

Bacillus ventricosus Heigener, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 102. 1935.

From Porter, 1937; Bredemann .... *B. brevis* No. 641

In 1902, Weiss (*177, p. 233*) described a species with the same name that was apparently quite different from the above. Under the rules of nomenclature (*16, p. 300*) the name used by Heigener is invalid.

IDENTIFICATION OF UNNAMED CULTURES

The following unnamed cultures were identified as *Bacillus brevis* during the course of this work.

Unnamed cultures	*B. brevis* No.
From Dubos, Rockefeller Inst., Med. Res., 1939, from soil	751
From Dubos, 1940, from Oka cheese	779
From Dubos, 1940, from Turkish cheese	780
From Dubos, 1940, from sewage	781
From Allison, USDA, 1940, a laboratory contaminant	782
From Lamanna, Wash. (State) Col., 1941, No. B40	799
From Bondi, Temple Univ., 1942, strains Collabella and Schotte	1027 and 1028
From Townsend and Zuch, Calif. Univ. and NCA, 1943, strain A (*166*)	1060
From Erdman, Nitragin Co., Milwaukee, 1945	1127
From Reuszer, USDA, 1946	1202–1214
From Rose, USDA, 1947	1222
From Bohrer, NCA, 1947, Nos. 11773–2 and 11773–8	T135 and T136
From Benedict, USDA, 1950, No. X64	1334
From Stanier, Calif. Univ., 1951, Nos. 6–9, 12, and 13	1359–1362, 1365, and 1366

Six strains of *B. brevis* were isolated from soil by the writers (Nos. 376–380 and 761).

GROUP 3. SPORANGIA SWOLLEN BY ROUND SPORES

Bacillus pantothenticus

Bacillus pantothenticus Proom and Knight, Jour. Gen. Microbiol. 4: 539. 1950.

By incubating soil suspensions in 4-percent NaCl, Knight and Proom (*84*) isolated 11 strains of a mesophilic sporeformer from the soils of widely separated localities in southern England. The new species was given its name because of its nutritional requirement of pantothenic acid. *Bacillus pantothenticus* also required aneurin (thiamine) and biotin in a medium containing salts and amino acids. In addition to the characters given below there was marked liquefaction of Loeffler's blood serum and a positive, restricted egg-yolk (lecithinase) reaction.

Morphologically *B. pantothenticus* was unique, owing to the formation of both round and oval spores. Because of the round spores it was placed in group 3, although in a natural classification it would seem to be intermediate between groups 2 and 3.

The following characterization was based on the examination of six authentic type strains (incubation temperature, 33° to 37° C., unless otherwise stated).

CHARACTERS

**Vegetative rods.*—0.4μ to 0.7μ by 1.2μ to 3.5μ; evenly stained; not in chains; motile; Gram-positive.

**Sporangia.*—Definitely swollen; drumstick- to racket-shaped.

**Spores.*—0.8μ to 1.0μ by 0.8μ to 1.3μ; round to oval; terminal; thin-walled. (Round spores appeared smaller than the oval.)

Colonies.—Small; round; translucent to dense; granular to moire; smooth to rough; embedded colonies lenticular or irregular and hairy.

Nutrient agar slants.—Growth moderate; thick; nonspreading; whitish.

Nutrient broth.—Light to moderate, uniform turbidity.

**NaCl broth.*—Growth in 10-percent concentration of NaCl; marked stimulation by 5-percent concentration.

**Glucose-nutrient agar slants.*—Growth scant; less than on nutrient agar.

Utilization of citrate.—Variable.

Potato.—No growth.

**Soybean agar slants.*—No growth.

Proteose-peptone acid agar slants.—No growth.

**Hydrolysis of starch.*—Positive.

**Production of acetylmethylcarbinol.*—Negative (incubation temperature 37° C.).

**pH of glucose broth.*—5.8 to 6.0.

**Fermentation tests.*—Acid without gas from glucose and sucrose; variable on arabinose; negative on xylose and mannitol. (Proom and Knight reported acid from arabinose and xylose with organic basal medium.)

Crystalline dextrins.—Negative.

**Hydrolysis of gelatin.*—Positive; wide zone of hydrolysis at 3 days.

**Hydrolysis of casein.*—Positive; wide zone of hydrolysis.

Production of indole.—Negative.

Urease.—Negative.

Reduction of nitrate to nitrite.—Variable.

Anaerobic production of gas from nitrate.—Negative. *Variation:* Small bubble of gas in two cases.

Anaerobic growth in glucose broth.—Scant growth; pH 5.4 to 6.4 at 14 days.

Reduction of methylene blue.—Variable.

Temperatures for growth.—Good growth at 33° to 40° C.; maximum temperature for grow 45° to 50°; slow growth at 28°.

TYPE CULTURES STUDIED

The following cultures of *Bacillus pantothenticus* were examined.

Bacillus pantothenticus

Nos. 1317–1322, from Proom, Wellcome Physiol. Res. Labs., England, 1950, Nos. CN 3020–CN 3023, CN 3028, and CN 3043.

Bacillus sphaericus

Bacillus sphaericus Neide, Centbl. f. Bakt. [etc.] Abt. 2, 12: 350. 1904.

In 1904 Neide gave the name of *Bacillus sphaericus* to a round, terminal-spored organism. Later, Ford (*93, p. 520*) considered Neide's organism identical with *B. pseudotetanicus* (Kruse) Migula (*115, p. 626*) and, because of priority, recommended the latter name. Kruse's culture, however, was said to be aerobic at ordinary temperature but to produce spores only at higher temperatures under anaerobic conditions. There is, therefore, some doubt as to whether Neide's and Kruse's cultures were the same. The situation is further complicated by the fact that Migula gave the name *B. pseudotetani* to an anaerobic gas-forming organism (*115, p. 598*). Apparently both names have been applied to the aerobic organism, as shown in the assignment of named cultures below and under *B. pumilus.*

Gottheil (*68*) introduced a round-spored species, *B. fusiformis,* in 1901, thus antedating Neide's *B. sphaericus* by 3 years. The descriptions of both these species were practically the same, with only a slight difference in the size of the spores and in the liquefaction of gelatin. Lawrence and Ford (*94, p. 312*) considered *B. fusiformis* identical with *B. lactimorbus.* (See Synonymy below.) Unfortunately, the name *B. fusiformis* had been applied to a strict anaerobe by Viellon and Zuber (*173*) in 1898. In accordance with the rules of nomenclature (*16, p. 304*), therefore, the writers recommend that *B. pseudotetanicus, B. pseudotetani,* and *B. fusiformis* be considered *nomina rejicienda* and that *B. sphaericus* be *nomen conservandum.*

The indications given by the original accounts that *B. sphaericus* and *B. fusiformis* were practically the same were borne out by the examination of cultures of both species. In 1946 (*147*) *B. fusiformis* was tentatively allocated as a variety of *B. sphaericus* and separated from it only by the production of urease. On the basis of a more detailed study of additional cultures, the urease-positive strains are now considered merely as representing a biotype of *B. sphaericus* and the formation of urease as a variable character of the species.

This change in classification has been strengthened by the studies of Knight and Proom (*84*) on the nutrition of *B. sphaericus* and of var. *fusiformis.* Six of 9 strains of *B. sphaericus* grew on casein basal medium with added aneurin alone. The remaining 3 strains and 10 of var. *fusiformis* required also the addition of biotin, neither aneurin nor biotin being effective singly. There was, therefore, no correlation between the nutritional requirements and the production of urease. Colmer (*34*), McGaughey and Chu (*110*), and Knight and Proom (*84*) found the egg-yolk (lecithinase) reaction of *B. sphaericus* and var. *fusiformis* to be negative.

In 1946 the writers (*147*) tentatively allocated *B. rotans* Roberts (*134*) and B. *loehnisii* Gibson (*61*) as varieties of *B. sphaericus,* pending study of more strains of each species. Unfortunately, no additional cultures were obtained. The decision had to be made, therefore, whether to characterize the varieties again on the basis of a single strain or to place the strains among the unclassified. The writers chose the latter alternative. There is no doubt that both *B. rotans* and *B. loehnisii* are very closely related to *B. sphaericus*, but without studying more cultures it would be impossible to determine whether they are distinct species or merely varieties or biotypes of *B. sphaericus*.

Bacillus sphaericus was inactive physiologically, its morphology and negative characters determining its identity for the most part. The following description of the species was based on 5 type cultures, 5 cultures bearing names in synonymy, 4 having other names, 27 unnamed cultures, and 5 isolations made by the writers: a total of 46 strains.

CHARACTERS

**Vegetative rods.*—Variable in size; 0.6μ to 1.0μ by 1.0μ to 7.0μ; ends rounded or pointed; motile; Gram-variable, often Gram-negative with Gram-positive granules.

On glucose-nutrient agar, cells contained a few small fat globules.

**Sporangia.*—Definitely bulged; usually drumstick-shaped. (See pl. 4, *F*.)

**Spores.*—0.7μ to 1.2μ in diameter; round; terminal to subterminal; spore wall usually thick and stainable; remnants of sporangia often adhering and making the surface rough and spiny; immature spores sometimes oval, becoming round; sporulation variable, best on soybean agar.

Colonies.—Thin; smooth; translucent; rapidly spreading over entire plate. *Variation:* Small; round; or irregular.

Giant colonies.—Usually motile micro-colonies moving in large arcs from point of inoculation; of various shapes; plate usually covered in 1 day. (Poured agar plates for this demonstration (*146*) were allowed to stand at room temperature for 2 or 3 days before use to dry the surface.)

Nutrient agar slants.—Growth thin; smooth; spreading; translucent. *Variations:* Rough; restricted; opaque; or wrinkled.

**Growth on nutrient agar slants, pH 6.0.*—Positive.

Nutrient broth.—Turbidity heavy; uniform to granular; sometimes with fragile pellicle.

**NaCl broth.*—Growth in 4-percent concentration of NaCl. *Variation:* No growth by four strains.

**Glucose-nutrient agar slants.*—Growth same as on nutrient agar.

Utilization of citrate.—Usually negative.

Potato.—Growth thin; soft; spreading; gray, usually becoming brownish with age.

**Soybean agar slants.*—Growth heavier and sporulation better than on nutrient agar.

Glucose-nitrate agar slants.—Scant, if any, growth.

**Hydrolysis of starch.*—Negative.

Production of acetylmethylcarbinol.—Negative (incubation temperature 32° C.).

**pH of glucose broth.*—7.8 to 8.2.

**Fermentation tests.*—Negative.

Hydrolysis of gelatin.—Usually positive.

Hydrolysis of casein.—Variable.

Production of indole.—Negative.

Urease.—Variable; approximately 50 percent of strains positive.

Reduction of nitrate to nitrite.—Negative.

Anaerobic production of gas from nitrate.—Negative.

Anaerobic growth in glucose broth.—Negative.

Reduction of methylene blue.—Positive; not reoxidized in 21 days.

Temperatures for growth.—Good growth at 28° to 35° C.; maximum temperature for growth 40° to 45°.

TYPE CULTURES STUDIED

The following type cultures of *Bacillus sphaericus* were received.

Bacillus sphaericus

No. 344, from ATCC, 1936, No. 94; AMNH No. 471; Ford.
No. 348, from ATCC, 1936, No. 4525; Ford No. 25.
Nos. 966 and 967, from Porter, Iowa Univ., 1940; Claussen Nos. 1911 and 1913.
No. T156, from Mauer, Army Med. Center, Washington, D. C., 1947.

From Porter, 1940; Bredemann. See *B. subtilis* No. 963.

SYNONYMY

Named strains that conformed to their original descriptions were identified as *Bacillus sphaericus* as follows.

Bacillus fusiformis Gottheil, Centbl. f. Bakt. [etc.] Abt. 2, 7: 724. 1901.

	B. sphaericus No.
From NCTC, 1937, No. 2608; Ford No. 24D	339
From AMNH, 1920, No. 732	350
From Porter, Iowa Univ., 1940; NCTC No. 2608; Ford No. 24D	866

For a discussion of this species and its present allocation, see introduction to *B. sphaericus* above.

From AMNH, 1920, contaminant of No. 350. See *B. pumilus* No. 247.
From ATCC, 1939, No. 61; Ford No. 10. See *B. circulans* No. 727 and *B. pumilus* No. 727A.
From ATCC, 1939, No. 4515; Ford No. 24. See *B. circulans* No. 728.
From Porter, 1940; Bredemann. See *B. pumilus* No. 864.
From Porter, 1940: Stapp. See *B. pumilus* No. 865.
From Porter, 1940; Claussen. See *B. cereus* No. 867.

Bacillus lactimorbus Jordan and Harris, Amer. Med. Assoc. Jour. 50: 1665. 1908.

From ATCC, 1939, No. 245 .... *B. sphaericus* No. 732

Jordan and Harris isolated this organism from the viscera of two heifers and a horse affected with milk sickness, or trembles, and called it *B. lactimorbi.* It was weakly pathogenic and for a time was thought to be the causative agent. Later Sackett (*136*) refuted this by showing that the cause of the disease was the eating of a poisonous plant.

From ATCC, 1939, No. 246; Jordan. See *B. cereus* No. 733.

Bacillus serositidis Lacorte, Inst. Oswaldo Cruz Mem. 26: 1. 1932. (English summary, p. 10.)

From Lacorte, Inst. Oswaldo Cruz, Brazil, 1937 .... *B. sphaericus* No. 668

ASSIGNMENT OF OTHER NAMED CULTURES

The following named cultures that did not correspond to their original descriptions were identified as *Bacillus sphaericus*.

Bacillus alvei

From Porter, Iowa Univ., 1940; Bredemann; Neide ...... *B. sphaericus* No. 810

Bacillus circulans

From Steinhaus, USPHS, 1942 .... *B. sphaericus* No. 1023

This strain was isolated by Steinhaus (*156, pp. 765 and 782*) from the alimentary canal of a Cecropia moth larva (*Samia cecropia* (L)).

Bacillus pseudotetani (Tavel) Migula, Syst. der Bakt., p. 598. 1900.
Pseudotetanus bacillus Tavel, Centbl. f. Bakt. [etc.] Originale (1) 23: 538. 1898.

From Tittsler, USDA, 1949 .... *B. sphaericus* No. 719

See the discussion of *B. sphaericus* above.

Bacillus simplex

From ATCC, 1936, No. 4526 .... *B. sphaericus* No. 349

See assigned cultures under *B. megaterium*.

IDENTIFICATION OF UNNAMED CULTURES

The following unnamed cultures were received and identified as *Bacillus sphaericus*.

Unnamed cultures

	B. sphaericus No.
From NIH, 1937	592 and 593
From Lamanna, Wash. (State) Col., 1941, No. A20	800
From Hansen, USDA, 1944	1089 and 1090
From Reuszer, USDA, 1946	1184–1201
From Bohrer, NCA, 1947, No. M–7	T111
From Rose, USDA, 1947, No. 6A	1223
From Delaporte, Lab. Cytol. Vég., Paris, 1949, No. 1003	1307
From Stanier, Calif. Univ., 1951, No. 11	1364

Four strains of *B. sphaericus* (Nos. 250, 400, 718, and 752) were isolated from soil by the writers and one (No. 717) from the intestine of an angleworm.

Bacillus pasteurii

Bacillus pasteurii (Miquel) Migula, Syst. der Bakt., p. 726. 1900.
Urobacillus pasteurii Miquel, Ann. de Micrographie 1: 552. 1889; 2: 13. 1890.

For some time the inability to grow on ordinary mediums without the addition of urea was generally thought to be the most important character for the identification of *Bacillus pasteurii*. In 1934, however, Gibson (*60*) pointed out that for many strains ammonia could be substituted for urea, provided the reaction of the medium was alkaline. After a study of 10 strains of *B. pasteurii*, Knight and Proom (*84*) reported that their nutritional requirements "were rather more heterogeneous than in most of the other species" of the genus. Four of the 10 cultures grew in a casein hydrolysate medium (with essential metabolites) at a pH of 8.5, whereas the remaining 6 required the addition of ammonia. Aneurin plus biotin or nicotinic acid was essential for the growth of all strains. There was no correlation between the need for ammonia and that for biotin or nicotinic acid. All strains, however, grew better when ammonia, aneurin, biotin, and nicotinic acid were added to the casein hydrolysate.

The following description of *B. pasteurii* was based on the study of four strains, two of which were used by Löhnis and Kuntze (*106*) during their investigation of urea-decomposing organisms. As no growth occurred at the ordinary neutral pH, 1 percent urea was added to all mediums. A 15-percent solution was sterilized by filtration and combined asceptically in appropriate quantities.

CHARACTERS

**Vegetative rods.*—0.6µ to 0.9µ by 1.5µ to 3.5µ; usually not in chains; ends rounded; motile; Gram-variable.

**Sporangia.*—Usually bulged.

**Spores.*—1.0µ to 1.2µ in diameter; round; terminal to subterminal.

Colonies.—Small; round; not distinctive.

Nutrient agar slants.—Growth thin; restricted; translucent.

Nutrient broth.—Turbidity uniform; moderate to heavy.

NaCl broth.—Growth in 10-percent concentration of NaCl.

Soybean agar slants.—Good growth; heavier than on nutrient agar.

Hydrolysis of starch.—Negative.

Production of acetylmethylcarbinol.—Negative (incubation temperature 32° C.).

Fermentation tests.—No growth on carbohydrate basal medium; on nutrient agar plus urea and the carbohydrate, acidity, if any, masked by decomposition of urea.

Hydrolysis of gelatin.—Positive.

Hydrolysis of casein.—Variable.

Urease.—Strongly positive.

Reduction of nitrate to nitrite.—Positive.

Anaerobic production of gas from nitrate.—Variable.

Anaerobic growth in glucose broth.—Positive; pH 8.6 after 14 days.

Temperatures for growth.—Good growth at 28° to 35° C.; maximum temperature for growth 37° to 40°.

TYPE CULTURES STUDIED

The following type cultures were examined.

Bacillus pasteurii

No. 673, from Gibson, Edinburgh Univ., Scotland, 1937, No. 22.
No. 674, from Löhnis, Leipzig Univ., Germany, 1914; Kral.
No. 675, from Löhnis, 1914, strain HAI.
No. 929, from Porter, Iowa Univ., 1940; Gibson No. 22.

UNCLASSIFIED CULTURES

Twenty strains bearing species names have not been assigned to any group or species. Some of them showed very few positive physiological characters and appeared to be weakened or aberrant cultures. It may be possible that the enlargement of the various species patterns by more reliable characters or means of rejuvenating these cultures will eventually permit their allocation. Other more physiologically active strains appeared to be entirely distinct. They may, perhaps, be representatives of species not included in this study or they may, on the other hand, belong to species inadequately described because of lack of sufficient number of cultures. More complete characterization, allowing for the natural variations of the species, may, therefore, include these now apparently unrelated strains.

Bacillus agrestis Werner, Zentbl. f. Bakt. [etc.] Abt. 2, 87: 468. 1933.

From Porter, Iowa Univ., 1937;
Bredemann .... Unclassified No. 602

Werner's description gives little information on the true identity of his organism. Culture No. 602 utilized mannitol and was lysed by the *Bacillus megaterium* bacteriophage. It failed, however, to make a good growth on glucose-nitrate agar, soybean agar, and potato and to utilize xylose and arabinose. In 1946 (*147*) this strain was assigned to the *B. megaterium* intermediates, but further study convinced the writers that it was a weakened strain instead, with too few remaining positive characters for definite identification.

Bacillus aminovorans Dooren de Jong, Zentbl. f. Bakt. [etc.] Abt. 2, 71: 215. 1927.

From NCTC, 1937, No. 2870;
Dooren de Jong .... Unclassified No. 341

Dooren de Jong isolated this strain from an enrichment culture of garden soil in a mineral solution containing trimethylamine. It was described as being unable to attack glucose, although a positive reading was recorded in one table, and as relatively inactive on the amines with the exception of methylamine.

Culture No. 341 was a Gram-positive, large rod (1.1μ to 1.6μ by 2.0μ to 5.0μ), granular in appearance, and somewhat resembling *B. megaterium*. The spores were round or nearly so and did not swell the rod appreciably. Growth on ordinary mediums was enhanced by the addition of tyrosine, cystine, or alanine. Glucose was the only carbohydrate utilized in the basal medium; starch was hydrolyzed; urease was produced; gelatin was weakly attacked, but casein was not; and its maximum temperature for growth was about 37° C.

Bacillus aporrhoeus Fuller and Norman, Jour. Bact. 46: 277. 1943.

From ATCC, 1944, No. 9500; Fuller . . Unclassified No. 1108
From Fuller, Iowa State Col., 1945 . . . Unclassified No. 1135

These cultures were isolated from soil and found to decompose polysaccharides, including cellulose. Morphologically and physiologically cultures Nos. 1108 and 1135 most closely resembled *B. circulans* except for the inhibition of their growth by glucose and the production of acetoin by No. 1135.

Bacillus badius Batchelor, Jour. Bact. 4: 25. 1919.

From Henry, Wash. (State) Univ., 1937, No. 110 . . . . Unclassified No. 663

Culture No. 663 seemed to agree with its original description. In type of growth it was similar to a partially dissociated culture of *B. cereus* var. *mycoides*. Microscopically it resembled a large-celled strain of *B. subtilis*. Carbohydrates were not hydrolyzed in the presence of organic or inorganic nitrogen; gelatin and casein were readily attacked; and the maximum temperature for growth was 52° C.

Bacillus cirroflagellosus ZoBell and Upham, Scripps Inst. Oceanography Bul. 5: 266. 1944.

From ZoBell, Scripps Inst., 1945, No. 563 . . . . Unclassified No. 1116

This strain was morphologically intermediate between groups 1 and 2. Physiologically it was very weak and required a concentration of 3-percent NaCl for optimum growth. Higher percentages of NaCl were inhibitive.

Bacillus cubensis Stührk, Zentbl. f. Bakt. [etc.] Abt. 2, 93: 192. 1935.

From Porter, 1940; Bredemann . . . . Unclassified No. 842

Morphologically strain No. 842 belonged to group 2. It was very weak physiologically, grew slowly, formed only a few spores, and required organic nitrogen. In 1946 the writers (*147*) listed it under *B. circulans*. Subsequent work, however, revealed so many negative characters that it was reassigned to the unclassified cultures.

Bacillus epiphytus ZoBell and Upham, Scripps Inst. Oceanography Bul. 5: 266. 1944.

From ZoBell, Scripps Inst., 1945, No. 567 . . . . Unclassified No. 1117

Morphologically and physiologically culture No. 1117 resembled *B. firmus* except for its utilization of citrate and anaerobic production of gas from nitrate.

Bacillus filicolonicus ZoBell and Upham, Scripps Inst. Oceanography Bul. 5: 270. 1944.

From ZoBell, Scripps Inst., 1945,
No. 585 .... Unclassified No. 1118

Culture No. 1118 morphologically belonged to group 2 and possibly to *B. circulans.* Its physiology, however, was too weak to permit definite assignment.

Bacillus flexus Batchelor, Jour. Bact. 4: 32. 1919.

From Henry, Wash. (State) Univ., 1937,
No. 131 .... Unclassified No. 665

This strain resembled *B. megaterium* microscopically, but failed to ferment xylose and arabinose, grew poorly on glucose-nitrate agar and potato, and was not lysed by the *B. megaterium* bacteriophage.

Bacillus freudenreichii (Miquel) Migula, Syst. der Bakt., p. 726. 1900.

Urobacillus freudenreichii Miquel, Ann. de Micrographie 2: 367, 488. 1890; Miquel and Cambier, Traite de Bact., p. 578. 1902.

From Gibson, Edinburgh Univ., Scotland, 1937,
No. 68 .... Unclassified No. 671

Gibson (*61, p. 493*) reviewed the literature on *B. freudenreichii* and concluded that it belonged to the *B. pasteurii* group. No authentic historical culture of the species was available for examination.

Morphologically culture No. 671 was similar to *B. pumilus.* It was, however, unable to ferment carbohydrates and was strongly positive for urease.

Bacillus loehnisii Gibson, Jour. Bact. 29: 495. 1935.

From Gibson, Edinburgh Univ., Scotland, 1937,
No. 97 .... Unclassified No. 672

Gibson considered *B. loehnisii* closely related to *B. pasteurii,* but differing in its ability to grow in ordinary neutral mediums. Only one strain of this species was available to the writers for study. Culture No. 672 differed from *B. sphaericus* only in its inability to grow at pH 6.0 or below. In 1946 the writers (*147*) assigned this culture to *B. sphaericus* as a variety. Upon reconsideration, however, it was decided not to allocate culture No. 672 until more strains and more data could be obtained.

Bacillus nondiastaticus Bergey and others, Jour. Bact. 4: 304. 1919.

From Porter, 1940; N. Y. [Geneva] Agr. Expt. Sta.
No. BG9 .... Unclassified No. 917

The original culture of *B. nondiastaticus* was described as being unable to attack starch. As culture No. 917 hydrolyzed starch readily, it was not considered authentic. It was not classified, because it appeared to possess some of the characters of *B. coagulans* and some of *B. stearothermophilus.*

Bacillus rarerepertus Schieblich, Zentbl. f. Bakt. [etc.] Originale (1), 124: 274. 1932.

From Porter, 1940; Schieblich ....Unclassified No. 943

Bacillus rarerepertus, an isolation from turnip leaves, was described as growing on all ordinary mediums with 37° C. as its optimum temperature for cultivation. Its rods measured 1.1μ to 1.5μ by 1.2μ to 4.5μ and developed round spores. Culture No. 943 was much smaller, with oval spores. Because of its refusal to grow on most mediums, its allocation was not attempted.

Bacillus rotans Roberts, Jour. Bact. 29: 234. 1935.

From Porter, 1937; Lewis; Roberts....Unclassified No. 633

Roberts was the first to see and describe motile colonies as produced by *B. rotans.* Later, Smith and Clark (*146*) noted the same phenomenon in cultures of *B. alvei, B. circulans,* and an unidentified Gram-negative rod, demonstrating that motile colonies were not limited to one species or to one genus.

Although Roberts made a number of isolations from the intestines of termites and from soil, only one strain (No. 633) was available to the writers for study. It differed from *B. sphaericus* in having a lower maximum temperature for growth (35° C.) and in being unable to grow at pH 6.0. In 1946 the writers (*147*) arbitrarily listed this strain as a variety of *B. sphaericus,* but later decided, as in the case of *B. loehnisii,* to postpone its allocation until more cultures and information could be obtained.

Bacillus simplex Gottheil, Centbl. f. Bakt. [etc.] Abt. 2, 7: 685. 1901.

From NCTC, 1937, No. 2597;
  Ford No. 11 ....Unclassified No. 335
From ATCC, 1936, No. 99; AMNH No. 737;
  Ford; Kral ....Unclassified No. 346
From Porter, 1940, NCTC, No. 2597;
  Ford No. 11 ....Unclassified No. 961
From Porter, 1940; Claussen; Blau....Unclassified No. 962

According to Gottheil, the vegetative rods of this species measured 0.9μ by 2.0μ to 3.0μ, with some long filaments, and the spores 0.83μ by 1.39μ to 2.2μ. Chester (*25, p. 84*) found that acid was not formed in glucose broth and that alkalinity increased in lactose and sucrose broths. He thought it was related to *B. subtilis.* Lawrence and Ford (*94, p. 310*) reported smaller measurements (0.56μ to 0.75μ by 3.0μ to 4.5μ) and that glucose broth was acidified. Many shadow-forms were noted, as well as the slowness of sporulation.

Of the above cultures, No. 346, weak in 1916 (*94, p. 310*), had lost entirely its ability to form spores, whereas Nos. 335, 961, and

962 developed a few spores on soybean agar, none on nutrient agar. They conformed roughly to the original measurements of Gottheil. The growth consisted of long tangled filaments and shadow-forms, which, with the lack or slowness of sporulation, indicated a deterioration of the cultures. All four cultures utilized glucose but not mannitol, were V-P negative, and did not grow anaerobically in the presence of glucose. There is a possibility that they are degenerate strains of *B. cereus,* but their lack of positive reactions makes any identification uncertain.

From ATCC, 1936, No. 4526. See *B. sphaericus* No. 349.
From Porter, 1940; Bredemann. See *B. subtilis* No. 959.
From Porter, 1940; Edwards; Conn. See *B. megaterium* No. 960.

Bacillus teres Neide, Centbl. f. Bakt. [etc.] Abt. 2, 12: 161. 1904.

From Porter, 1940; Stapp .... Unclassified No. 986

Neide listed *B. teres* in the synonymy of *B. albolactis* (*B. cereus* in the present work) and also included *B. globigii* (*B. licheniformis* in the present work) in the same group. Culture No. 986 was listed in 1946 (*147*) as a *B. megaterium-B. cereus* intermediate. Reconsideration, however, led to the conclusion that its positive characteristics were too few to permit positive identification.

From Porter, 1937; Bredemann. See *B. pumilus* No. 637.
From Porter, 1940; Claussen. See *B. subtilis* No. 987.

LITERATURE CITED

(1) AMERICAN PUBLIC HEALTH ASSOCIATION AND AMERICAN WATER WORKS ASSOCIATION.
1946. STANDARD METHODS FOR THE EXAMINATION OF WATER AND SEWAGE. Ed. 9, 286 pp., illus. New York.

(2) AYERS, S. H., and JOHNSON, W. T., JR.
1924. STUDIES OF THE STREPTOCOCCI. VII. A MEDIUM FOR STOCK CULTURES OF STREPTOCOCCI AND OTHER BACTERIA. Jour. Bact. 9: 111–114.

(3) ——— RUPP, P., and JOHNSON, W. T., JR.
1919. A STUDY OF THE ALKALI-FORMING BACTERIA FOUND IN MILK. U. S. Dept. Agr. Bul. 782, 39 pp., illus.

(4) BABERS, F. H.
1938. A SEPTICEMIA OF THE SOUTHERN ARMYWORM CAUSED BY BACILLUS CEREUS. Ent. Soc. Amer. Ann. 31: 371–373.

(5) BANERJEA, R.
1944. SOME OBSERVATIONS ON VOGES-PROSKAUER TEST. Indian Jour. Med. Res. 32: 15–18.

(6) BARRITT, M. M.
1936. THE INTENSIFICATION OF THE VOGES-PROSKAUER REACTION BY THE ADDITION OF α-NAPHTHOL. Jour. Path. and Bact. 42: 441–454.

(7) ———
1943. NOTES ON THE α-NAPHTHOL MODIFICATION OF THE VOGES-PROSKAUER TEST AND REFERENCE TO THE CREATINE MODIFICATION. Jour. Hyg. [London] 43: 214–216.

(8) BATTY-SMITH, C. G.
1941. THE DETECTION OF ACETYL-METHYL-CARBINOL IN BACTERIAL CULTURES. Jour. Hyg. [London] 41: 521–529.

(9) BECKER, M. E., and PEDERSON, C. S.
1950. THE PHYSIOLOGICAL CHARACTERS OF BACILLUS COAGULANS (BACILLUS THERMOACIDURANS). Jour. Bact. 59: 717–725.

(10) BEKKER, J. H.
1941. UEBER DEN ZUSAMMENHANG ZWISCHEN KAPSELBILDUNG HÄMODIGESTION UND VIRULENZBEIM MILZBRANDBAZILLUS. Zentbl. f. Bakt. [etc.] Originale (1) 147: 451–453.

(11) ———
1943. DIE HÄMODIGESTION DES MILZBRANDBAZILLUS. Zentbl. f. Bakt. [etc.] Originale (1) 150: 326–327.

(12) BENEDEK, T.
1927. SCHIZOSACCHAROMYCES HOMINIS NOV. SPEC., DIE ERSTE MENSCHENPATHOGENE SPALTHEFE. Centbl. f. Bakt. [etc.] Originale (1) 104: [291]–303, illus.

(13) BREED, R. S.
1929. SOME ADDITIONAL STORIES ABOUT SCIENTIFIC NAMES. Science 70: 480.

(14) ——— MURRAY, E. G. D., and HITCHENS, A. P.
1948. BERGEY'S MANUAL OF DETERMINATIVE BACTERIOLOGY. Ed. 6, 1529 pp. Baltimore.

(15) BRUNSTETTER, B. C., and MAGOON, C. A.
1932. STUDIES ON BACTERIAL SPORES. III. A CONTRIBUTION TO THE PHYSIOLOGY OF SPORE PRODUCTION IN BACILLUS MYCOIDES. Jour. Bact. 24: 85–122, illus.

(16) BUCHANAN, R. E., ST. JOHN-BROOKS, R., and BREED, R. S.
1948. INTERNATIONAL BACTERIOLOGICAL CODE OF NOMENCLATURE. Jour. Bact. 55: 287–306.

(17) BURDON, K. L.
1946. FATTY MATERIAL IN BACTERIA AND FUNGI REVEALED BY STAINING DRIED, FIXED SLIDE PREPARATIONS. Jour. Bact. 52: 665–678, illus.

(18) BURDON, K. L., STOKES, J. C., and KIMBROUGH, C. E.
1942. STUDIES OF THE COMMON AEROBIC SPORE-FORMING BACILLI. I. STAINING FOR FAT WITH SUDAN BLACK B-SAFRANIN. Jour. Bact. 43: 717–724, illus.
(19) BURNSIDE, C. E., and FOSTER, R. E.
1935. STUDIES ON THE BACTERIA ASSOCIATED WITH PARAFOULBROOD. Jour. Econ. Ent. 28: 578–584, illus.
(20) BURTON, L. V., and RETTGER, L. F.
1917. CORRELATIONS IN THE COLON-AEROGENES GROUP. Jour. Infect. Dis. 21: [162]–195, illus.
(21) CAMERON, E. J., and ESTY, J. R.
1926. THE EXAMINATION OF SPOILED CANNED FOODS. II. CLASSIFICATION OF FLAT SOUR, SPOILAGE ORGANISMS FROM NONACID FOODS. Jour. Infect. Dis. 39: [89]–105, illus.
(22) CARBONE, D., RAMAZZOTTI, G., MAZZUCCHI, M., and MONTI, P. C.
1921. LE REAZIONI SIERODIAGNOSTICHE DEI MICROORGANISMI BANALI. Ist. Sieroterap. Milan, Bol. 2: 29–46.
(23) CASMAN, E. P., and RETTGER, L. F.
1933. LIMITATION OF BACTERIAL GROWTH AT HIGHER TEMPERATURES. Jour. Bact. 26: 77–123.
(24) CHESTER, F. D.
1901. A MANUAL OF DETERMINATIVE BACTERIOLOGY. 401 pp., illus. New York and London.
(25) ———
1904. OBSERVATIONS ON AN IMPORTANT GROUP OF SOIL BACTERIA. ORGANISMS RELATED TO BACILLUS SUBTILIS. Del. Agr. Expt. Sta. Ann. Rpt. (1902–1903) 15: 42–96, illus.
(26) CHORINE, V.
1929. NEW BACTERIA PATHOGENIC TO THE LARVAE OF PYRAUSTA NUBILALIS HB. Internatl. Livestock Exposition [Chicago], Internatl. Corn Borer Invest. Sci. Rpts. 2: 39–53, illus.
(27) CHU, H. P.
1950. THE PRODUCTION OF NON-CAPSULATED AVIRULENT VARIANTS BY BACILLUS ANTHRACIS AND ITS IMPLICATION ON TAXONOMY. (Abstract) Jour. Gen. Microbiol. 4 (3): 13.
(28) CLARK, F. E.
1937. THE RELATION OF BACILLUS SIAMENSIS AND SIMILAR PATHOGENIC SPORE-FORMING BACTERIA TO BACILLUS CEREUS. Jour. Bact. 33: 435–443.
(29) ———
1939. NONMOTILE VARIANTS OF BACILLUS ALVEI. Jour. Bact. 38: 491–497.
(30) ——— and SMITH, N. R.
1938. CARBOHYDRATE FERMENTATION RESPONSES OF THE AEROBIC SPORE-FORMING BACILLI. Jour. Bact. (Abstract G17) 35: 9–10.
(31) ——— and SMITH, N. R.
1939. CULTURAL REQUIREMENTS FOR THE PRODUCTION OF BLACK PIGMENTS BY BACILLI. Jour. Bact. 37: 277–284.
(32) CLEVERDON, R. C., PELCZAR, M. J., JR., and DOETSCH, R. N.
1949. VITAMIN REQUIREMENTS OF BACILLUS COAGULANS. Jour. Bact. 58: 113–114.
(33) COBLENTZ, L. M.
1943. RAPID DETECTION OF THE PRODUCTION OF ACETYL-METHYL-CARBINOL. Amer. Jour. Pub. Health 33: 815–817.
(34) COLMER, A. R.
1948. THE ACTION OF BACILLUS CEREUS AND RELATED SPECIES ON THE LECITHIN COMPLEX OF EGG YOLK. Jour. Bact. 55: 777–785, illus.
(35) CONN, H. J.
1921. THE USE OF VARIOUS CULTURE MEDIA IN CHARACTERIZING ACTINOMYCETES. N. Y. State Agr. Expt. Sta. Tech. Bul. 83, 26 pp.

(36) CONN, H. J.
1930. THE IDENTITY OF BACILLUS SUBTILIS. Jour. Infect. Dis. 46: [341]–350, illus.

(37) ———
1936. BIOLOGICAL STAINS. Ed. 3, 276 pp., illus. Geneva, N. Y.

(38) ——— and BREED, R. S.
1919. THE USE OF THE NITRATE-REDUCTION TEST IN CHARACTERIZING BACTERIA. Jour. Bact. 4: 267–290.

(39) COURMONT, P., and ROCHAIX, A.
1923. VARIATIONS IN VITRO DE LA FERMENTATION DES "SUCRES" PAR LES BACILLES DU GROUPE DYSENTÉRIQUE. Soc. de Biol. [Paris] Compt. Rend. 88: 784–786.

(40) ——— and ROCHAIX, A.
1923. VARIATIONS DE LA FERMENTATION DES "SUCRES" PAR LES BACILLES DYSENTÉRIQUES SOUS L'INFLUENCE DU "CO-ENTRAINEMENT." Soc. de Biol. [Paris] Compt. Rend. 88: 786–788.

(41) CUNNINGHAM, A.
1931. THE LIFE CYCLE OF B. SACCHAROBUTYRICUS VON KLECKI. III. THE SLENDER ROD PHASE AND SECONDARY TRANSFORMATIONS. Zentbl. f. Bakt. [etc.] Abt. 2, 83: 1–21, illus.

(42) DAVIS, J. G., and ROGERS, H. J.
1939. THE EFFECT OF STERILIZATION UPON SUGARS. Zentbl. f. Bakt. [etc.] Abt. 2, 101: 102–110, illus.

(43) DESAI, S. V.
1932. STUDIES ON BACTERIOPHAGES OF THE ROOT NODULE ORGANISMS. Indian Jour. Agr. Sci. 2: 138–156, illus.

(44) DESMOTS, H.
1904. PRODUCTION DE L'ACÉTYLMÉTHYLCARBINOL PAR LES BACTÉRIES DU GROUPE DU BACILLUS MESENTERICUS. [Paris] Acad. des Sci. Compt. Rend. 138: 581–583.

(45) DOOREN DE JONG, L. E. DEN.
1931. STUDIEN ÜBER BAKTERIOPHAGIE. III. UEBER BAC. UNDULATUS N. SPEC. UND DEN DARIN ANWESENDEN BAKTERIOPHAGEN. Zentbl. f. Bakt. [etc.] Originale (1) 122: 277–286, illus.

(46) ———
1933. ÜBER BACILLUS MYCOIDES UND DEN PLEOMORPHISMUS. Arch. f. Mikrobiol. 4: 36–53, illus.

(47) DORNER, W., and HELLINGER, E.
1935. STUDIES ON THE VOGES-PROSKAUER TEST. (Abstract G18) Jour. Bact. 29: 16–17.

(48) DUBOS, R. J.
1940. THE ADAPTIVE PRODUCTION OF ENZYMES BY BACTERIA. Bact. Rev. 4: 1–16.

(49) ——— and HOTCHKISS, R. D.
1941. THE PRODUCTION OF BACTERICIDAL SUBSTANCES BY AEROBIC SPORULATING BACILLI. Jour. Expt. Med. 73: 629–640.

(50) DUCLAUX, E.
1894. LE LAIT, ÉTUDES CHIMIQUES ET MICROBIOLOGIQUES. Ed. 2, 376 pp., illus. Paris.

(51) FLÜGGE, C.
1886. DIE MIKROORGANISMEN. 692 pp., illus. Leipzig.

(52) ———
1894. DIE AUFGABEN UND LEISTUNGEN DER MILCHSTERILISIRUNG GEGENÜBER DEN DARMKRANKHEITEN DER SÄUGLINGE. Ztschr. f. Hyg. u. Infektionskrank. 17: 272–342, illus.

(53) ———
1896. DIE MIKROORGANISMEN. 2 v., illus. Leipzig.

(54) FRANCIS, A. E., and RIPPON, J. E.
1949. BACILLUS POLYMYXA AND ITS BACTERIOPHAGES. Jour. Gen. Microbiol. 3: 425–433, illus.

(55) Frankland, G. C., and Frankland, P. F.
1887. STUDIES ON SOME NEW MICROORGANISMS OBTAINED FROM AIR. Roy. Soc. London, Phil. Trans., Ser. B, 178: 257–287.

(56) Frazier, W. C.
1926. A METHOD FOR THE DETECTION OF CHANGES IN GELATIN DUE TO BACTERIA. Jour. Infect. Dis. 39: [302]–309, illus.

(57) Gause, G. F.
1939. SOME PHYSIOLOGICAL PROPERTIES OF DEXTRAL AND OF SINISTRAL FORMS IN BACILLUS MYCOIDES FLÜGGE. Biol. Bul. 76: 448–465, illus.

(58) ——— and Brazhnikova, M. G.
1944. GRAMICIDIN S. ORIGIN AND MODE OF ACTION. Lancet 247: 715–716.

(59) Georgi, C. E., and McMaster, M. E.
1944. INFLUENCE OF BACTERIOLOGIC PEPTONES ON HYDROGEN SULFIDE, INDOL AND ACETYL METHYL CARBINOL PRODUCTION BY ENTEROBACTERIACEAE. Soc. Expt. Biol. and Med. Proc. 55: 185–189, illus.

(60) Gibson, T.
1934. AN INVESTIGATION OF THE BACILLUS PASTEURI GROUP. II. SPECIAL PHYSIOLOGY OF THE ORGANISMS. Jour. Bact. 28: 313–322.

(61) ———
1935. AN INVESTIGATION OF THE BACILLUS PASTEURI GROUP. III. SYSTEMATIC RELATIONSHIPS OF THE GROUP. Jour. Bact. 29: 491–502.

(62) ———
1937. THE IDENTITY OF BACILLUS SUBTILIS AND ITS DIFFERENTIATION FROM OTHER SPORE-FORMING BACTERIA. Soc. Agr. Bact. Proc. Abs. 1937.

(63) ———
1944. A STUDY OF BACILLUS SUBTILIS AND RELATED ORGANISMS. Jour. Dairy Res. 13: 248–260, illus.

(64) ——— and Topping, L. E.
1938. FURTHER STUDIES OF THE AEROBIC SPORE-FORMING BACILLI. Soc. Agr. Bact. Proc. Abs. 1938: 43–44.

(65) Gordon, R. E., and Smith, N. R.
1949. AEROBIC SPOREFORMING BACTERIA CAPABLE OF GROWTH AT HIGH TEMPERATURES. Jour. Bact. 58: 327–341.

(66) Gorini, C.
1894. STUDI CRITICO-SPERIMENTALI SULLA STERILIZZAZIONE DEL LATTE. R. Univ. Pavia, Soc. Ital. d'Ig. Gior. 16: [5]–24.

(67) ———
1943. PER L'UNIFICAZIONE DEI FERMENTI LATTICI SPORIGENI. R. Ist. Lombardo di Sci. e Let. Rend., Cl. di Sci. Mat. e Nat., (ser. 3, v. 7) 76: [3]–8.

(68) Gottheil, O.
1901. BOTANISCHE BESCHREIBUNG EINIGER BODENBAKTERIEN. BEITRÄGE ZUR METHODE DER SPECIESBESTIMMUNG UND VORARBEIT FÜR DIE ENTSCHEIDUNG DER FRAGE NACH DER BEDEUTUNG DER BODENBAKTERIEN FÜR DIE LANDWIRTSCHAFT. Centbl. f. Bakt. [etc.] Abt. 2, 7: 430–435, [449]–465, [481]–497, [529]–544, 582–591, 627–637, 680–691, 717–730, illus.

(69) Graaf, H. de.
1941. A REVISION OF THE PROCEDURE FOR THE VOGES-PROSKAUER TEST. Antonie van Leeuwenhoek, Jour. Microbiol. Serol. 7: [92].

(70) Harden, A., and Norris, D.
1912. THE BACTERIAL PRODUCTION OF ACETYLMETHYLCARBINOL AND 2.3-BUTYLENE GLYCOL FROM VARIOUS SUBSTANCES. II. Roy. Soc. London, Proc., Ser. B., 85: 73–78.

(71) Hartman, T. L.
1940. THE USE OF SUDAN BLACK B AS A BACTERIAL FAT STAIN. Stain Technol. 15: 23–28, illus.

(72) HASTINGS, E. G.
1903. MILCHAGAR ALS MEDIUM ZUR DEMONSTRATION DER ERZEUGUNG PROTEOLYTISCHER ENZYME. Centbl. f. Bakt. [etc.] Abt. 2, 10: 384.

(73) HINSHELWOOD, C., and JACKSON, S.
1950. THE STABILITY OF *d*-ARABINOSE ADAPTATION OF BACT. LACTIS AEROGENES. Roy. Soc. London, Proc., Ser. B., 886: 88–95, illus.

(74) HIRSCHHORN, H. N., BUCCA, M. A., and THAYER, J. D.
1948. SUBTENOLIN. AN ANTIBIOTIC FROM BACILLUS SUBTILIS. I. BACTERIOLOGIC PROPERTIES. Soc. Expt. Biol. and Med. Proc. 67: 429–432.

(75) HOROWITZ-WLASSOWA, L. M., and RODIONOWA, E. A.
1932. ÜBER DIE AZETOÏNGÄRUNG. Zentbl. f. Bakt. [etc.] Abt. 2, 87: 333–339, illus.

(76) HUSSONG, R. V., and HAMMER, B. W.
1928. A THERMOPHILE COAGULATING MILK UNDER PRACTICAL CONDITIONS. Jour. Bact. 15: 179–188.

(77) IMŠENECKI, A.
1945. ON THE STRUCTURE OF ANAEROBIC BACTERIA. Jour. Bact. 49: 1–5.

(78) INTERNATIONAL ASSOCIATION OF MICROBIOLOGISTS.
1951. BACTERIOLOGICAL NOMENCLATURE AND TAXONOMY. OPINION 1. THE CORRECT SPELLING OF THE SPECIFIC EPITHET IN THE SPECIES NAME BACILLUS MEGATERIUM DE BARY 1884. Internatl. Bul. Bact. Nomencl. and Taxon. 1: 35–36.

(79) JANSEN, E. F., and HIRSCHMANN, D. J.
1944. SUBTILIN—AN ANTIBACTERIAL PRODUCT OF BACILLUS SUBTILIS, CULTURING CONDITIONS AND PROPERTIES. Arch. Biochem. 4: 297–309.

(80) JOHNSON, B. A., ANKER, H., and MELENEY, F. L.
1945. BACITRACIN: A NEW ANTIBIOTIC PRODUCED BY A MEMBER OF THE B. SUBTILIS GROUP. Science 102: 376–377.

(81) KATZNELSON, H., and LOCHHEAD, A. G.
1944. STUDIES WITH BACILLUS POLYMYXA. III. NUTRITIONAL REQUIREMENTS. Canadian Jour. Res., Sect. C, Bot. Sci. 22: [273]–279.

(82) ——— and LOCHHEAD, A. G.
1947. NUTRITIONAL REQUIREMENTS OF BACILLUS ALVEI AND BACILLUS PARA-ALVEI. Jour. Bact. 53: 83–88.

(83) KELLERMAN, K. F., and MCBETH, I. G.
1912. THE FERMENTATION OF CELLULOSE. Centbl. f. Bakt. [etc.] Abt. 2, 34: 485–494, illus.

(84) KNIGHT, B. C. J. G., and PROOM, H.
1950. A COMPARATIVE SURVEY OF THE NUTRITION AND PHYSIOLOGY OF MESOPHILIC SPECIES IN THE GENUS BACILLUS. Jour. Gen. Microbiol. 4: 508–538.

(85) KOCH, R.
1881. ZUR UNTERSUCHUNG VON PATHOGENEN ORGANISMEN. Mitt. aus dem K. Gesundheitsamte 1: 1–48.

(86) KOSER, S. A.
1924. CORRELATION OF CITRATE UTILIZATION BY MEMBERS OF THE COLON-AEROGENES GROUP WITH OTHER DIFFERENTIAL CHARACTERISTIC AND WITH HABITAT. Jour. Bact. 9: 59–77.

(87) ——— and VAUGHAN, E. F.
1937. A STUDY OF *d*-ARABINOSE FERMENTATION. Jour. Bact. 33: 587–602.

(88) KRISTENSEN, M.
1948. MUTATIVE BACTERIAL FERMENTATION. Acta Path. et Microbiol. Scand. 25: [244]–248.

(89) LAMANNA, C.
1940. THE TAXONOMY OF THE GENUS BACILLUS. II. DIFFERENTIATION OF SMALL CELLED SPECIES BY MEANS OF SPORE ANTIGENS. Jour. Infect. Dis. 67: [193]–204.

(90) Lamanna, C.
1940. THE TAXONOMY OF THE GENUS BACILLUS. III. DIFFERENTIATION OF THE LARGE CELLED SPECIES BY MEANS OF SPORE ANTIGENS. Jour. Infect. Dis. 67: [205]–212.

(91) ———
1942. THE STATUS OF BACILLUS SUBTILIS, INCLUDING A NOTE ON THE SEPARATION OF PRECIPITINOGENS FROM BACTERIAL SPORES. Jour. Bact. 44: 611–617.

(92) Landy, M., Warren, G. H., Rosenman, S. B., and Colio, L. G.
1948. BACILLOMYCIN: AN ANTIBIOTIC FROM BACILLUS SUBTILIS ACTIVE AGAINST PATHOGENIC FUNGI. Soc. Expt. Biol. and Med. Proc. 67: 539–541.

(93) Laubach, C. A., Rice, J. L., and Ford, W. W.
1916. STUDIES ON AEROBIC SPORE-BEARING NON-PATHOGENIC BACTERIA. II. Jour. Bact. 1: 493–533, illus.

(94) Lawrence, J. S., and Ford, W. W.
1916. STUDIES ON AEROBIC SPORE-BEARING NON-PATHOGENIC BACTERIA. I. Jour. Bact. 1: 273-316, illus.

(95) Lehmann, K. B., and Neumann, R. O.
1896. ATLAS UND GRUNDRISS DER BAKTERIOLOGIE. 2 v., illus. Munich.

(96) ——— and Neumann, R. O.
1931. DETERMINATIVE BACTERIOLOGY. II. 868 pp., illus. New York. [English translation of the 7th German edition. Breed, R. S., ed.]

(97) Lemoigne, M.
1913. ASSIMILATION DU SACCHAROSE PAR LES BACTÉRIES DU GROUPE DU "B. SUBTILLIS". FERMENTATION BUTYLÈNEGLYCOLIQUE. Inst. Pasteur [Paris], Ann. 27: [856]–885.

(98) ——— and Delaporte, B., and Croson, M.
1944. CONTRIBUTION A L'ÉTUDE BOTANIQUE ET BIOCHEMIQUE DES BACTÉRIES DU GENRE BACILLUS (PREMIER MÉMOIRE). VALEUR DU TEST DE L'ACÉTYLMÉTHYLCARBINOL POUR LA CARACTÉRISATION DES ESPÈCES. Inst. Pasteur [Paris], Ann. 70: [65]–79.

(99) ——— and Hooreman, M., and Croson, M.
1949. RECHERCHES SUR LA FORMATION D'ACÉTOINE, AUX DÉPENS DE L'ACIDE PYRUVIQUE ET L'ÉTHANOL PAR BACILLUS SUBTILIS. Inst. Pasteur [Paris], Ann. 76: [303]–313, illus.

(100) Levine, M.
1916. ON THE SIGNIFICANCE OF THE VOGES-PROSKAUER REACTION. Jour. Bact. 1: 153–164.

(101) ——— Buchanan, J. H., and Lease, G.
1927. EFFECT OF CONCENTRATION AND TEMPERATURE ON GERMICIDAL EFFICIENCY OF SODIUM HYDROXIDE. Iowa State Col. Jour. Sci. 1: 379–394, illus.

(102) Lewis, I. M.
1932. DISSOCIATION AND LIFE CYCLE OF BACILLUS MYCOIDES. Jour. Bact. 24: 381–[421], illus.

(103) ———
1934. BACTERIAL VARIATION WITH SPECIAL REFERENCE TO BEHAVIOR OF SOME MUTABILE STRAINS OF COLON BACTERIA IN SYNTHETIC MEDIA. Jour. Bact. 28: 619–[639], illus.

(104) ——— and Worley, G.
1936. BACTERIOPHAGY OF BACILLUS MYCOIDES WITH REFERENCE TO EFFECT ON DISSOCIATION AND TRANSMISSION BY SPORES. Jour. Bact. 32: 195–198.

(105) Lison, L.
1934. SUR DE NOUVEAUX COLORANTS HISTOLOGIQUES SPÉCIFIQUES DES LIPIDES. Soc. de Biol. [Paris] Compt. Rend. 115: 202–205.

(106) Löhnis, F., and Kuntze, W.
1908. BEITRÄGE ZUR KENNTNIS DER MIKROFLORA DES STALLDÜNGERS. Centbl. f. Bakt. [etc.] Abt. 2, 20: 676–687.

(107) LUTMAN, B. F., and WHEELER, H. E.
1948. BACILLUS MEGATERIUM DE BARY FROM THE INTERIOR OF HEALTHY POTATO TUBERS. Wash. Acad. Sci. Jour. 38: 336–340, illus.

(108) MCCOY, E. F.
1940. A SEROLOGICAL ANALYSIS OF A BACTERIAL POPULATION. Internatl. Cong. Microbiol. Proc. (1939) 3: 160–161.

(109) MCCRAY, A. H.
1917. SPORE-FORMING BACTERIA OF THE APIARY. Jour. Agr. Res. 8: 399–420, illus.

(110) MCGAUGHEY, C. A., and CHU, H. P.
1948. THE EGG-YOLK REACTION OF AEROBIC SPORING BACILLI. Jour. Gen. Microbiol. 2: 334–340, illus.

(111) MANNINGER, R., and NOGRADI, A.
1948. ÜBER INDUZIERTE MUTATIONSERSCHEINUNGEN AN MILZBRAND UND KARTOFFELBAZILLEN. Experientia 4: 276–277, illus.

(112) MATTES, O.
1927. PARASITÄRE KRANKHEITEN DER MEHLMOTTENLARVEN UND VERSUCHE ÜBER IHRE VERWENDBARKEIT ALS BIOLOGISCHES BEKÄMPFUNGSMITTEL. Gesell. f. Beförd. Gesam. Naturw. Sitzber. 62: [381]–417, illus.

(113) MICKELSON, M. N., and WERKMAN, C. H.
1939. PRESSURE-AERATION EFFECTS ON THE DISSIMILATION OF GLUCOSE BY AEROBACTER INDOLOGENES. Iowa State Col. Jour. Sci. 13: 157–160.

(114) MIGULA, W.
1897. ÜBER DEN ZELLINHALT VON BACILLUS OXALATICUS ZOPF. Arb. aus dem Bakt. Inst. Tech. Hochsch. zu Karlsruhe 1: [137]–147, illus.

(115) ———
1900. SYSTEM DER BAKTERIEN. 2 v., illus. Jena.

(116) MUSCHEL, A.
1922. ZUR CHEMIE DER SCHWARZFÄRBUNG KOHLENHYDRATHALTIGER NÄHRBÖDEN DURCH DEN BACILLUS MESENTERICUS VAR. NIGER. Biochem. Ztschr. 131: 570–590.

(117) NADSON, G. A., and ADAMOVIC, S. M.
1910. ÜBER DIE BEEINFLUSSUNG DER ENTWICKLUNG DES BACILLUS MYCOÏDES FLÜGGE DURCH SEINE STOFFWECHSELPRODUKTE. [Leningrad] Jard. Imper. Bot. Bul. 10: 154–165. St. Petersburg. [In Russian, German summary, pp. 164–165.]

(118) NÈGRE, L.
1913. BACTÉRIES THERMOPHILES DES SABLES DU SAHARA. Soc. de Biol. [Paris] Compt. Rend. 74: 814–816.

(119) NEIDE, E.
1904. BOTANISCHE BESCHREIBUNG EINIGER SPORENBILDENDEN BAKTERIEN. Centbl. f. Bakt. [etc.] Abt. 2, 12: [1]–32, [161]–176, [337]–352, 539–554, illus.

(120) NYBERG, C.
1927. MORPHOLOGIE DU BACILLUS MYCOÏDES. Finska Vetenks. Soc. Comm. Biol. 3 (3): [1]–22, illus.

(121) ———
1929. ZUR BIOLOGIE DES BACILLUS MYCOÏDES. I. DIE KOLONIETYPEN, WELCHE DURCH SOGENANNTE MUTATION AUS DEM BAC. MYCOÏDES ENTSTEHEN KÖNNEN. Soc. Med. Fenn. "Duodecim," Acta 12 (2): [3]–18, illus. Helsinki.

(122) OESTERLE, P., and STAHL, C. A.
1929. UNTERSUCHUNGEN ÜBER DEN FORMENWECHSEL UND DIE ENTWICKLUNGSFORMEN BEI BACILLUS MYCOIDES. Zentbl. f. Bakt. [etc.] Abt. 2, 79: 1–25, illus.

(123) O'MEARA, R. A. Q.
1931. A SIMPLE, DELICATE AND RAPID METHOD OF DETECTING THE FORMATION OF ACETYL METHYLCARBINOL BY BACTERIA FERMENTING CARBOHYDRATE. Jour. Path. and Bact. 34: 401–406.

(124) Pan, S. C., Peterson, W. H., and Johnson, M. J.
1940. ACCELERATION OF LACTIC ACID FERMENTATION BY HEAT-LABILE SUBSTANCES. Indus. and Engin. Chem., Indus. Ed., 32: 709–714, illus.

(125) Perlberger, J.
1924. ÜBER DIE FERMENTATIVE WIRKUNG DER GRUPPE DES B. MYCOIDES UND SEINER NÄCHSTEN VERWANDTEN AUF KOHLEHYDRATE NEBST EINIGEN BEMERKUNGEN ÜBER DIE MORPHOLOGIE DIESER GRUPPE. Centbl. f. Bakt. [etc.] Abt. 2, 62: [1]–15.

(126) Porter, R., McCleskey, C. S., and Levine, M.
1937. THE FACULTATIVE SPORULATING BACTERIA PRODUCING GAS FROM LACTOSE. Jour. Bact. 33: 163–183.

(127) Price, C. W., Nielsen, J. K., and Welch, H.
1946. THE ESTIMATION OF STREPTOMYCIN IN BODY FLUIDS. Science 103: 56–57.

(128) Prickett, P. S.
1928. THERMOPHILIC AND THERMODURIC MICROORGANISMS WITH SPECIAL REFERENCE TO SPECIES ISOLATED FROM MILK. V. DESCRIPTION OF SPOREFORMING TYPES. N. Y. State Agr. Expt. Sta. Tech. Bul. 147, 58 pp.

(129) Pringsheim, E. G., and Langer, J.
1924. ZUR ENTWICKLUNGSPHYSIOLOGIE DER KOLONIEN DES BACILLUS MYCOIDES FLÜGGE. Centbl. f. Bakt. [etc.] Abt. 2, 61: [225]–246, illus.

(130) Rautenstein, J. I.
1946. DISSOCIATION OF BAC. MYCOIDES. I. MORPHOLOGY OF VARIANTS. Microbiology [Moscow] 15: [291]–301, illus. [In Russian, English summary, p. 301.]

(131) Reynolds, H., and Werkman, C. H.
1937. CARBOLIGATIC ACTIVITY OF ESCHERICHIA COLI. Arch. f. Mikrobiol. 8: 149–152.

(132) Rippel, A.
1940. DIE SCHREIBWEISE VON BACILLUS MEGATERIUM DE BARY. Arch. f. Mikrobiol. 11: [470].

(133) Robbins, W. J., and Ma, R.
1941. BIOTIN AND THE GROWTH OF FUSARIUM AVENACEUM. Torrey Bot. Club Bul. 68: 446–462, illus.

(134) Roberts, J. L.
1935. A NEW SPECIES OF THE GENUS BACILLUS EXHIBITING MOBILE COLONIES ON THE SURFACE OF NUTRIENT AGAR. Jour. Bact. 29: 229–234, illus.

(135) Russ-Münzer, A.
1938. UEBER EINEN EIGENARTIGEN BEWEGUNGSTYPUS SCHWÄRMENDER BAKTERIEN. Zentbl. f. Bakt. [etc.] Originale (1) 142: 175–178.

(136) Sackett, W. G.
1919. THE CONNECTION OF MILKSICKNESS WITH THE POISONOUS QUALITIES OF WHITE SNAKE-ROOT (EUPATORIUM URTICAEFOLIUM). Jour. Infect. Dis. 24: 231–259.

(137) St. John-Brooks, R., and Breed, R. S.
1937. ACTIONS TAKEN BY THE SECOND INTERNATIONAL MICROBIOLOGICAL CONGRESS IN LONDON, 1936, REGARDING BACTERIOLOGICAL NOMENCLATURE. Jour. Bact. 33: 445–447.

(138) Salmoiraghi, G. C.
1947. AZIONE DEI FOSFATI E DEL GLUCOSIO NELLA FERMENTAZIONE BUTILENGLICOLICA. Soc. Ital. di Biol. Sper. Bol. 23: 31–32.

(139) ———
1947. UN PROCEDIMENTO SEMPLICE PER DIMOSTRERE LA PRODUZIONE DI ACETILMETILCARBINOLO DA PARTE DEI MICROBI. Riv. dell'Ist Sieroterap. Ital., Sez. 1, 22: [121]–125, illus.

(140) Schmidt, C. F.
1950. spore formation by thermophillic flat sour organisms. i. the effect of nutrient concentration and the presence of salts. Jour. Bact. 60: 205–212.

(141) Schweiger, L. B., Trainer, D., and Eveleth, D. F.
1943. isolations of bacillus subtilis from actinobacillosis-like lesions in cattle and sheep. Amer. Jour. Vet. Res. 4: 127–133, illus.

(142) Sievers, O., and Zetterberg, B.
1940. a preliminary investigation into the antigenic characters of spore-forming, aerobic bacteria. Jour. Bact. 40: 45–56.

(143) Silverman, M., and Werkman, C. H.
1941. the formation of acetylmethylcarbinol from pyruvic acid by a bacterial enzyme preparation. Jour. Biol. Chem. 138: 35–48, illus.

(144) Siribaed, L.
1935. b. siamensis, a pathogenic variety of b. subtilis. Jour. Infect. Dis. 57: [143]–146.

(145) Smith, N. R., and Clark, F. E.
1937. a proposed grouping of the mesophilic, aerobic, spore-forming bacilli. Soil Sci. Soc. Amer. Proc. 2: 255.

(146) ——— and Clark, F. E.
1938. motile colonies of bacillus alvei and other bacteria. (Abstract A4) Jour. Bact. 35: 59–60.

(147) ——— Gordon, R. E., and Clark, F. E.
1946. aerobic mesophilic sporeforming bacteria. U. S. Dept. Agr. Misc. Pub. 559, 112 pp., illus.

(148) Smith, R. F. W., and Baker, J. L.
1898. bacillus luteus sporogenes. Centbl. f. Bakt. [etc.] Abt. 2, 4: 788–789, illus.

(149) Snieszko, S., and Skorka, J.
1939. the effect of temperature on results secured with some modifications of the voges-proskauer test. Jour. Bact. 38: 682–683.

(150) Society of American Bacteriologists, Committee on Bacteriological Technic.
1951. manual of methods for pure culture study of bacteria. leaflet v. routine tests for the descriptive chart. 12th ed., 24 pp., illus. H. J. Conn, ed. Geneva, N. Y.

(151) Soriano, S., and Soriano, A. M. de.
1940. the influence of variation on the classification of bacterial species. Internatl. Cong. Microbiol. Proc. (1939) 3. 157–158. New York.

(152) Stahly, G. L., and Werkman, C. H.
1942. origin and relationship of acetylmethylcarbinol to 2.3-butylene glycol in bacterial fermentations. Biochem. Jour. 36: 575–581, illus.

(153) Stansly, P. G., and Schlosser, M. E.
1947. studies on polymyxin: isolation and identification of bacillus polymyxa and differentiation of polymyxin from certain known antibiotics. Jour. Bact. 54: 549–556.

(154) Stapp, C., and Zycha, H.
1931. morphologische untersuchungen an bacillus mycoides; ein beitrag zur frage des pleomorphismus der bakterien. Arch. f. Mikrobiol. 2: [493]–536, illus.

(154a) Stark, E., and Tetrault, P. A.
1952. a determinative study of amylolytic, stenothermophilic bacteria isolated from soil. Sci. Agr. 32: 81–92, illus.

(155) Stein, C. D.
1943. studies and observations on the laboratory diagnosis of anthrax. Vet. Med. [Chicago] 38: 130–139, illus.

(156) Steinhaus, E. A.
1941. A STUDY OF THE BACTERIA ASSOCIATED WITH THIRTY SPECIES OF INSECTS. Jour. Bact. 42: 757–790.

(157) ———
1951. POSSIBLE USE OF BACILLUS THURINGIENSIS BERLINER AS AN AID IN THE BIOLOGICAL CONTROL OF THE ALFALFA CATERPILLAR. Hilgardia 20: 359–381, illus.

(158) Stern, R. M., Hegarty, C. P., and Williams, O. B.
1942. DETECTION OF BACILLUS THERMOACIDURANS (BERRY) IN TOMATO JUICE, AND SUCCESSFUL CULTIVATION OF THE ORGANISM IN THE LABORATORY. Food Res. 7: 186–191.

(159) Sterne, M.
1937. VARIATION IN BACILLUS ANTHRACIS. Onderstepoort Jour. Vet. Sci. and Anim. Indus. 8: 271–349.

(160) Stotz, E., and Raborg, J.
1943. A COLORIMETRIC DETERMINATION OF ACETOIN AND DIACETYL. Jour. Biol. Chem. 150: 25–31, illus.

(161) Tarr, H. L. A.
1936. BACILLUS ALVEI AND BACILLUS PARA-ALVEI. Zentbl. f. Bakt. [etc.] Abt. 2, 94: 509–511, illus.

(162) Thornton, H. G.
1922. ON THE DEVELOPMENT OF A STANDARDIZED AGAR MEDIUM FOR COUNTING SOIL BACTERIA, WITH ESPECIAL REGARD TO THE REPRESSION OF SPREADING COLONIES. Ann. Appl. Biol. 9: 241–274, illus.

(163) Tilden, E. B., and Hudson, C. S.
1942. PREPARATION AND PROPERTIES OF THE AMYLASES PRODUCED BY BACILLUS MACERANS AND BACILLUS POLYMYXA. Jour. Bact. 43: 527–544, illus.

(164) Tomcsik, J.
1949. ZUR FRAGE DER INDUZIERTEN MUTATION DER MILZBRANDBAZILLEN. Schweiz. Ztschr. f. Path. und Bakt. 12: [489]–499, illus.

(165) ———
1950. INDUCED MUTATION OF BACILLUS ANTHRACIS. (Abstract) Jour. Gen. Microbiol. 4 (3): [16]–[17].

(166) Townsend, C. T., and Zuch, T. L.
1943. STUDIES ON AGAR CONTAMINATION AS AFFECTING THE STERILIZATION OF CULTURE MEDIA. Jour. Bact. 46: 269–271.

(167) Trevisan, V. B. A.
1889. I GENERI E LE SPECIE DELLE BATTERIACEE. 36 pp., illus. Milan.

(168) Tsen, E. T. H., and Sung, S.
1932. SOYBEAN CULTURE MEDIA. China Med. Jour. 46: 603–606.

(169) Turner, A. W., and Eales, C. E.
1941. AN AEROBIC SPORULATING BACILLUS THAT FORMS ROTATING AND MIGRATING COLONIES. Austral. Jour. Expt. Biol. and Med. Sci. 19: [161]–166, illus.

(170) Vaughn, R., Mitchell, N., and Levine, M.
1938. EFFECT OF TEMPERATURE AND TEST REAGENTS ON THE VOGES-PROSKAUER AND METHYL RED REACTIONS. (Abstract A19) Jour. Bact. 36: 313–314.

(171) ——— Mitchell, N., and Levine, M.
1939. THE VOGES-PROSKAUER AND METHYL RED REACTIONS IN THE COLI-AEROGENES GROUP. Amer. Water Works Assoc. Jour. 31: 993–1001.

(172) ——— Wedding, G. T., and Tabachnick, J.
1948. THE VOGES-PROSKAUER REACTION AND DIFFERENTATION OF THE COLIFORM BACTERIA. Hilgardia 18: 357–368, illus.

(173) Viellon and Zuber.
1898. RECHERCHES SUR QUELQUES MICROBES STRICTEMENT ANAËROBIES ET LEUR ROLE EN PATHOLOGIE. Arch. de Méd. Expt. et Anat. Path. [Paris], Ser. 1, 10: [517]–545.

(174) Voges, O., and Proskauer, B.
1898. BEITRAG ZUR ERNÄHRUNGSPHYSIOLOGIE UND ZUR DIFFERENTIALDIAGNOSE DER BAKTERIEN DER HÄMORRHAGISCHEN SEPTICÄMIE. Ztschr. f. Hyg. u. Infektionskrank. 28: 20–32.

(175) Webley, D. M.
1947. ACTIVITY OF THERMOPHILIC BACTERIA IN COMPOSTS OF FRESH GREEN MATERIAL. Nature [London] 159: 35, illus.

(176) Weigmann, H.
1898. UEBER ZWEI AN DER KÄSEREIFUNG BETEILIGTE BAKTERIEN. Centbl. f. Bakt. [etc.] Abt. 2, 4: 820–834, illus.

(177) Weiss, R.
1902. ÜBER DIE BAKTERIENFLORA DER SAUREN GÄHRUNG EINIGER NAHRUNGS UND GENUSSMITTEL. Arb. aus dem Bakt. Inst. Tech. Hochsch. zu Karlsruhe 2: [165]–272, illus.

(178) Wernig, H.
1940. ZUR KENNTNIS DER BEDINGUNGEN DER VOGES-PROSKAUERSCHEN REAKTION UND DES VORKOMMENS VON AEROGENESBAZILLEN BEI DER CYSTOPYELITIS. Zentbl. f. Bakt. [etc.] Originale (1) 145: 270–285, illus.

(179) White, G. F.
1912. THE CAUSE OF EUROPEAN FOUL BROOD. U. S. Bur. Ent. Cir. 157, 15 pp., illus.

(180) Withers, W. A., and Ray, B. J.
1911. MODIFICATION OF THE DIPHENYLAMINE TEST FOR NITROUS AND NITRIC ACIDS. N. C. Agr. Expt. Sta. Ann. Rpt. 1910: 56–58.

(181) Zopf, W.
1883. DIE SPALTPILZE. 100 pp., illus. Breslau.

(182) ———
1885. DIE SPALTPILZE. Ed. 3. 127 pp., illus. Breslau.

INDEX TO NAMED CULTURES STUDIED

An index of the named cultures of the genus *Bacillus* that were studied in this work, together with their identifications, is given below. If a culture failed to conform to the original description of the species whose name it bore or if the original account was too vague to permit recognition, its identification has been placed in parentheses, and it may be found in the list of the assigned cultures of the species. The identifications not in parentheses indicate that the cultures bearing those particular names were thought to be authentic and are either in the list of type cultures or in the synonymy of the species.

Numerals refer to the number of the culture in the writers' collection.

Name as received	Identification
Bacillus	*Bacillus*
abysseus	(*licheniformis* 1114)
acetoethylicum	*macerans* 278
adhaerens Laubach	(*pumilus* 309 and 337)
adhaerens Stührk	(*subtilis* 802)
aegyptiacus	(*brevis* 803)
aerosporus	*polymyxa* 1237
aethylicus	(*cereus* 804)
agrestis	Unclassified 602
agri	(*cereus* 310 and 342; *pumilus* 336; *brevis* 1219)
agrophilus	(*pumilus* 807)
albolactis	*cereus* 721 and 808
alpinus	(*brevis* 603)
alvei	*alvei* 662, 683, 684, and 811; (*sphaericus* 810)
aminovorans	Unclassified 341
amylolyticus	*circulans* 290
anthracis	*cereus* var. *anthracis* 1007–1020
aporrhoeus	Unclassified 1108 and 1135
asterosporus	*polymyxa* 251, 280, 812, and 813
aterrimus	*subtilis* 263, 330, 652, and 814; *subtilis* var. *aterrimus* 653; (*pumilus* 266; *megaterium* 267; *cereus* 722 and 723)
badius	Unclassified 663
bellus	(*brevis* 605)
betainovorans	(*brevis* 606)
betanigrificans	*macerans* 646 and 649
borborokoites	(*subtilis* 1115)
borstelensis	(*brevis* 818)
brevis	*brevis* 604 and 1137–1139; (*pumilus* 724 and 725)
butyricus	(*subtilis* 820)
calidolactis	(*licheniformis* 821; *stearothermophilus* T15 and T16; *coagulans* T2006–T2008)
capri	*megaterium* 607, 822. and 824
carotarum	*megaterium* 608, 828, and 829; (*circulans* 826; *pumilus* 827)
centrosporus	*brevis* 664
cereus	*cereus* 232, 305, 1122, 1130, and 1256
cereus var. *fluorescens*	*cereus* 645
circulans	*circulans* 358, 726. 831, 1136, and 1280–1282; (*sphaericus* 1023)
cirroflagellosus	Unclassified 1116

Name as received	Identification
closteroides	*circulans* 832
coagulans	*coagulans* 609, 795–798, and T13; (*cereus* 833)
cobayae	*megaterium* 610, 835, and 837
cohaerens	(*pumilus* 334; *circulans* 838A and 839A; *megaterium* 838B and 839B)
consolidus	(*circulans* 841)
cubensis	Unclassified 842
danicus	*megaterium* 245 and 246; (*subtilis* 601)
dendroides	(*pumilus* 844)
dentatus	(*brevis* 611)
dextrolacticus	*coagulans* 784
effluens	*circulans* 1250
elegans	(*brevis* 612)
ellenbachensis	*cereus* 847–851
endoparasiticus	(*megaterium* 1103, 1224, and 1225; *subtilis* 1107 and 1248; *cereus* 1249)
epiphytus	Unclassified 1117
fastidiosus	(*pumilus* 643)
filicolonicus	Unclassified 1118
filiformis	(*licheniformis* 778)
firmus	*firmus* 613, 854, 855, 858, and 860; (*brevis* 856, 857, and 859)
flavidus	(*firmus* 861)
flexus	Unclassified 665
formosus	(*brevis* 614)
freudenreichii	Unclassified 671
fusiformis	*sphaericus* 339, 350, and 866; (*pumilus* 247, 727A, 864, and 865; *circulans* 727 and 728; *cereus* 867)
geniculatus	(*subtilis* 772)
globigii	*licheniformis* 1289–1292; (*circulans* 313; *pumilus* 331; *subtilis* var. *niger* 1221A and 1221B)
goniosporus	*cereus* 767
granularis	(*brevis* 870)
graveolens	*megaterium* 615 and 872; (*subtilis* 730)
hollandicus	*brevis* 616 and 874; (*cereus* 875)
immobilis	(*megaterium* 1024)
imomarinus	*firmus* 1119
kaustophilus	*stearothermophilus* T81
kellermanii	*circulans* 1251
krzemieniewski	*circulans* 760
lacticola	*cereus* 617, 731, and 877; (*pumilus* 878)
lactimorbus	*sphaericus* 732; (*cereus* 733)
lactis	*cereus* 618 and 879–881
lactis niger	*subtilis* var. *niger* 254 and 655; *subtilis* 255; (*cereus* 256 and 654)
lasiocampa	(*cereus* 1092)
laterosporus	*laterosporus* 314, 340, 347, 590, and 882; (*cereus* 589)
lautus	(*circulans* 666, mixed with *cereus*)
lentus	*lentus* 670, 883, and 1262
licheniformis	*licheniformis* 1263–1265
limnophilus	(*brevis* 887)
liodermis	(*pumilus* 734)
loehnisii	Unclassified 672
luteus	(*subtilis* 619; *pumilus* 885; *cereus* 886)
macerans	*macerans* 277, 888, 1093, and 1095–1101
maculatus	(*brevis* 889)
malabarensis	*megaterium* 239; (*pumilus* 620 and 891)

Name as received	Identification
megaterium	*megaterium* 234, 308, and 892–895 (*pumilus* 735 and 736; *subtilis* 737)
mesentericus	*pumilus* 236, 307, 738, and 896
mesentericus flavus	*pumilus* 333 and 739
mesentericus fuscus	*subtilis* 897 and 898
mesentericus hydrolyticus	*subtilis* 901
mesentericus ruber	*licheniformis* 900; (*cereus* 644)
mesentericus viscosus	(*cereus* 903)
metiens	*cereus* 621
minimus	(*subtilis* 773)
modestus	(*coagulans* 905)
montanus	(*brevis* 622)
musculi	*megaterium* 623 and 907; (*subtilis* 909)
mycoides	*cereus* 1266; *cereus* var. *mycoides* 233, 306, 911, 912, and 1316; (*brevis* 910)
natto	*subtilis* 351 and 352
niger	*subtilis* var. *niger* 220, 264, 650, and 651; (*pumilus* 657 and 658; *licheniformis* 913)
nigrificans	*subtilis* var. *aterrimus* 624, 659, and 740; (*pumilus* 647)
nitidus	(*circulans* 625)
nitroxus	(*licheniformis* 918)
nondiastaticus	Unclassified 917
orae	(*subtilis* 919)
orpheus	*laterosporus* 661, 681, and 682
oxalaticus	*megaterium* 627 and 923
oxalactis	(*cereus* 922)
pabuli	(*circulans* 924)
pallidus	(*brevis* 628)
panis	*subtilis* 315, 332, and 926; (*pumilus* 316)
pantothenticus	*pantothenticus* 1317–1322
para-alvei	*alvei* 685, 686, and 1141
parvus	*subtilis* 1106; (*pumilus* 629 and 928)
pasteurii	*pasteurii* 673–675 and 929
petasites	*megaterium* 343 and 931; (*subtilis* 338, 930, and 933)
petroselini	*cereus* 934
polymyxa	*polymyxa* 279, 354, 1105, 1238, 1312–1314, and T2010
praussnitzii	*cereus* 935; *cereus* var. *mycoides* 936
pseudotetani	(*sphaericus* 719)
pseudotetanicus	(*pumilus* 937)
pulvifaciens	*pulvifaciens* 1283–1285
pumilus	*pumilus* 272, 630, 939, and 940; (*subtilis* 741 and 941; *cereus* 942)
rarerepertus	Unclassified 943
rarus	(*brevis* 632)
robur	*cereus* 946 and 947; (*subtilis* 631)
rotans	Unclassified 633
rufescens	(*brevis* 948)
rugulosus	(*subtilis* 949)
ruminatus	*megaterium* 951 and 952; (*pumilus* 345; *subtilis* 634)
santiagensis	(*brevis* 953)
scaber	(*subtilis* 774 and 775)
schuylkilliensis	*macerans* 1030 (*circulans* 1029A; *brevis* 1029B)
segetalis	(*brevis* 635)
serositidis	*sphaericus* 668
serrulatus	(*licheniformis* 955)
sessilis	*cereus* 768

Name as received	Identification
siamensis	*cereus* 201
silvaticus	*megaterium* 957 and 958; (*subtilis* 636)
simplex	Unclassified 335, 346, 961, and 962; (*sphaericus* 349; *subtilis* 959; *megaterium* 960)
soli	*macerans* 1252
sphaericus	*sphaericus* 344, 348, 966, 967, and T156; (*subtilis* 963)
stearothermophilus	*stearothermophilus* T17, T19, T85, T91, T92, and T2004
sublustris	(*pumilus* 980)
submarinus	(*subtilis* 1120)
subtilis	*subtilis* 231, 743, 744, 968, 969, 971, 972, 975, 1088, 1109, 1125, 1126, 1286–1288, and 1315; (*pumilus* 1299–1301)
subtilis (Ford)	*licheniformis* 243, 304, 560, 745, 978, 1128, and 1323–1327
subtilis (Michigan)	*cereus* 303, 537, 538, 970, 973, 974, and 976
subtilis niger	*subtilis* var. *niger* 704
subtilis var. *viscosus*	*subtilis* 979
supraresistens	(*circulans* 977)
tardivus	(*subtilis* 981)
tenuis	*subtilis* 777; (*licheniformis* 776)
teres	Unclassified 986; (*pumilus* 637; *subtilis* 987)
terminalis	(*circulans* 746; *subtilis* 983)
terminalis var. *thermophilus*	(*pumilus* 984)
terrestris	(*pumilus* 638)
thalassokoites	(*subtilis* 1121)
thermoacidificans	*coagulans* T186 and T2011
*thermoaciduran*s	*coagulans* 770, T21–T27, T2005, and T2021–T2023
thermophilus	(*pumilus* 982 and T84)
thuringiensis	*cereus* var. *thuringiensis* 996
torquens	*circulans* 1253 and 1254
tritus	(*pumilus* 667)
tropicus	*cereus* 793
truffauti	(*pumilus* 355 and 997)
tumescens	*megaterium* 991, 994, 995, and 1102; (*subtilis* 989; *pumilus* 990: *cereus* 992 and 993)
tyrosinogenes	(*subtilis* var. *aterrimus* 353)
undulatus	*cereus* 998 and 999
vagans	*macerans* 1255
validus	(*circulans* 639)
valinovorans	(*brevis* 640)
ventricosus	(*brevis* 641)
vulgatus	*subtilis* 237, 238, 787, 1003, and 1004; (*licheniformis* 329)
vulgatus hydrolyticus	*subtilis* 747
watzmannii	(*subtilis* 642)
Lactobacillus	
cereale	*coagulans* T2012 and T2013
delbrueckii	(*coagulans* T2020)

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